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1-Pentacosanol Isolated from Stem Ethanolic Extract of *Cayratia trifolia* (L.) is A Potential Target for Prostate Cancer-*In SILICO* Approach

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Abstract

Cayratia trifolia (L.) are the traditional medicinal plants used in the Indian Ayurvedic system of medicine. The main objective of the study was to isolate and characterize the structure and function of bioactive compound from ethanolic extract of stem parts of *Cayratia trifolia* (L.) against prostate cancer targets such as PTEN, AKT, SMO and E2F3 by *in silico* approach. Column, Thin layer chromatography, UV-visible spectrophotometer (UV), Fourier Transform Infrared (FTIR), ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy suggested that the isolated natural bioactive compound probably like 1-pentacosanol. The molecular docking results revealed that AKT, E2F3, PTEN and SMO complex with 1-pentacosanol have good glide score of -3.428, -3.573, -3.964 and -3.987 Kcal/mol and the glide energy is -36.846, -31.761, -39.270 and -34.919 Kcal/mol respectively when compared with standard drug, i.e. Finasteride (complex with AKT, E2F3, PTEN and SMO (no interaction) has low glide score and glide energy -3.1/22.168, -3.8/-41.588 and -3.1/-40.050 Kcal/mol, respectively. The ADME property of the isolated natural compound of 1-pentacosanol was under acceptable range. Based on the results, it can be concluded that the isolated 1-pentacosanol compound may act as novel inhibitors against prostate cancer targets.

Keywords: *Cayratia trifolia*, Chromatography techniques, NMR studies, 1-pentacosanol, Molecular docking. ADME properties.

1. Introduction

Cancer is associated with multiple genetic and regulatory aberrations in the cell. It is a highly heterogeneous disease, both morphologically and genetically (Yan *et al.,* 2007). Prostate cancer is the second most common malignancy (after lung cancer) in men worldwide, counting 1,276,106 new cases and instigating 358,989 deaths (3.8% of all deaths caused by cancer in men) in 2018 (Bray *et al.,* 2018). Prostate cancer is an assorted disease visible in varying pathological and clinical forms. It is complicated to diagnose and treat as prostate cancer tumors may be detected only during autopsy. Significant advancement has been achieved in prostate cancer diagnosis with the introduction of prostate-specific antigen (PSA) screening (Wang *et al.,* 1979). Structural biology and balanced drug design, proteomics and cell imaging contain major role in understanding receptor and drug interactions in prostate cancer (Reynolds, 2008).

Analysis of cancer pathways shows a number of interrelated markers responsible for oncogenesis. The recent studies suggest that, Phosphatase and tensin homolog (PTEN), Protein kinase B (AKT), Smoothened (SMO) and E2F3 overexpression and amplification have central roles in the initiation, progression and metastasis of prostate cancer (Pradip and William, 2005; Feng *et al.,* 2007; Mehrian *et al.,* 2007; Sinosh *et al.,* 2010). A large proportion of the world population depends on the traditional medicine because of the shortage and high expenses of orthodox medicine (Perumal *et al.,* 2014), compared with synthetic compounds, natural products provide inherent larger-scale diversity and have been the major resource of bioactive agents for new drug discovery. From the point of view of research, natural products are rapidly being utilized as source for drug discovery and development (Poornima *et al.,* 2014). From 2003-2012, 22 and 14 natural bioactive compounds having potent antitumor activity, which were isolated from marine fungi and marine red algae respectively (Pejin *et al.,* 2013; Pejin *et al.,* 2015).

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Cayratia trifolia (L.) is the medicinal plant which belongs to the family of Vitaceae, and it has been reported to contain huge number of bioactive compounds such as yellow waxy oil, steroids, terpenoids, flavonoids and tannins (Gupta and Sharma, 2007; Gupta *et al.,* 2012). Whole plant is used in the treatment of tumors, neuralgia, hepatic problems (Guru kumar *et al.*, 2011). This plant extract has also been reported to have antibacterial, antioxidants, antiviral, antiprotozoal, hypoglycaemic activity etc (Kumar *et al.*, 2012; Sowmya *et al.,* 2014, Perumal *et al.,* 2015,). Therefore, the aim of the present study is to isolate, structurally characterize and analyze the anti-prostate cancer potential of isolated compound from stem ethanolic extract of *Cayratia trifolia*.

2. Materials and Methods

2.1. Plant collection

The stem parts of *Cayratia trifolia* (L.) were collected from and around the area of Kumbakonam, Tamil Nadu, India and authenticated by Dr. P. Sathyanarayanan, Botanical survey of India, Tamil Nadu Agricultural University Campus, Coimbatore. The voucher number is BSI/SRC/5/23/2010-2011/Tech.1527 (Perumal *et al.*, 2012). The fresh stem plant material was washed under the running tap water, dipped on saline overnight, air dried and finely powdered for further use.

2.2. Extract preparation

200 g of powdered plant material was weighed and extracted with 1000 ml of ethanol for 72 hours using occasional shaker. The supernatant was collected and concentrated at 40°C in reduced pressure using a rotary evaporator. The dried extract was stored at 4°C for further study.

2.3. Isolation and Identification of bioactive compound

2.3.1. Column chromatography

Chromatographic techniques are based on separation of substances between a stationary and a mobile phase. The mobile phase moves relative to the stationary one. Components of a mixture to be separated move together, with the mobile phase due to their different interactions with the phases. The column chromatography (4 ×100 cm) was performed using 60-120 mesh silica gel to elute out individual compounds from the stem parts of ethanolic extract. After loading with the plant extract (5 g) mixed with 10-20 g of activated silica gel and the column was run with varying solvent polarities with different ratios like Petroleum ether (100%), Petroleum ether: Chloroform (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9), Chloroform (100%), Chloroform**:** Ethyl acetate (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9), Ethyl acetate (100%), Ethyl acetate**:** Methanol (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9) and Methanol (100%). The fractions were collected and tested by Thin Layer Chromatography (TLC) for single spot.

2.3.2. Thin layer chromatography

Thin layer chromatography is an easy and highly useful technique in research laboratories to separate and identify unknown compounds. It is used for the separation of a mixture into individual components using a stationary and mobile phase (Sadasivam and Manikam, 2004).

The optimized conditions were used for the identification of active constituents present in the plant extract. The fractions collected from chromatographic columns were monitored by TLC in different solvent systems. These plates were placed in the solvent chamber containing mobile phase. The solvent was allowed to rise to the maximum height of the TLC plate, then they were removed from solvent chamber, dried and the spots were detected by placing the TLC plates in a chamber containing iodine vapour. Fractions identified single spots in iodine chamber, R_f value was calculated and pooled together and proceeded for further analysis.

2.4. Functional group analysis and structure characterization

2.4.1. UV-Visible Spectroscopy

The UV-Visible spectroscopy offers a simple, cheap and easy-to-use technique to identify and quantify the main phytochemicals in relation to the polarity of the extraction solvent (Zavoi *et al*., 2011). Each isolated fraction was determined using the UV region (200-400nm) and visible region (400-800nm) using the UV-Vis-2450, Shimadzu instrument.

2.4.2. Fourier Transform Infrared (FTIR) spectroscopy

FTIR has proven to be a valuable tool for the characterization and identification of compounds or functional groups (chemical bonds) present in an unknown mixture of plants extract (Eberhardt *et al*., 2007). In addition, FTIR spectra of pure compounds are usually unique, acting as a "molecular fingerprint" (Hazra *et al*., 2007).

The Shimadzu FTIR Spectrum instrument consists of globar and mercury vapour lamp as sources, an interferometer chamber comprising of KBr and Mylar beam splitters followed by a sample chamber and detector. Entire region of 450-4000 cm^{-1} is covered by this instrument. The spectrometer works under purged conditions. Solid samples are dispersed in KBr or polyethylene pellets depending on the region of interest. This instrument has a typical resolution of 1.0 cm^{-1}. Signal averaging, signal enhancement, base line correction and other spectral manipulations are possible.

2.4.3. Nuclear Magnetic Resonance (NMR) spectroscopy

NMR spectroscopy is used to determine the molecular structure based on the chemical environment of the magnetic nuclei like ^{1}H, ^{13}C, 2D NMR etc., even at low concentrations. This is one of the most powerful non-destructive techniques in elucidating the molecular structure of biological and chemical compounds and used in organic chemistry, biology, medicine, pharmaceuticals, etc., for characterization of compounds. This technique is used in JEOL GSX 400 NB FT-NMR spectrometer. The spectra of samples containing low abundant nuclei like ^{1}H, ^{13}C, etc. are thus easily obtained. Also, dynamic studies are possible by relaxation measurements. Homo and hetero ^{1}H decoupling are also possible.

2.5. In silico analysis

The 3D structure of AKT (PDB ID: 4GV1), E2F3, PTEN (PDB ID: 1D5R) and SMO (PDB ID: 4QIM) was retrieved from the Protein Data Bank (www.rcsb.org), and proteins were prepared by protein preparation wizards (standard methods) that are available in grid-based ligand

docking with energetic (Protein Preparation Wizard, 2012). The active site (binding pocket) and functional residues of AKT, E2F3, PTEN and SMO were identified and characterized by site-map module from Schrodinger package. The isolated bioactive compound was used in molecular docking studies. These ligands were prepared using the LigPrep 2.4. The structure of each ligands was optimized. All docking analysis were performed by using the standard precision (SP) which is Standard mode of Glide (Grid based Ligand Docking with Energetic) module from Schrodinger 2012. The isolated bioactive compound was docked in to

the binding site AKT, E2F3, PTEN and SMO using GLIDE. ADME properties predictions were carried out using QikProp 2.3 module. QikProp helps in analysing the pharmacokinetics and pharmacodynamics of the ligand by accessing the drug like properties. Significant ADME properties such as molecular weight (MW), H-bond donor, H-bond acceptor and log P (O/W) were predicted.

3. Results

Column chromatography was performed in the stem ethanolic extract of *Cayratia trifolia* (L.) by varying solvent polarities with different ratios like Petroleum ether (100%), Petroleum ether : Chloroform (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9), Chloroform (100%), Chloroform **:** Ethyl acetate (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9), Ethyl acetate (100%), Ethyl acetate **:** Methanol (9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9) and Methanol (100%). Totally 223 fractions were collected and analysed by TLC which is shown in table 1.

Table 1. Isolated fractions of stem ethanolic extract of *Cayratia trifolia* Separation of fractions by column chromatography

Solvents	Ratio	Fractions collected
Petroleum ether	(100%)	1-20
Petroleum ether: Chloroform	9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9	21-30, 31-37, 38-45, 56-58, 49-55, 56-60, 61-69, 76-80
Chloroform	100%	81-109
Chloroform: Ethyl acetate	9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9	110-116, 117-120, 121-125, 126-129, 130-132, 133-137, 138-147, 148-150, 151-153
Ethyl acetate	100%	154-160
Ethyl acetate: Methanol	9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9	161-163, 164-169, 170-178, 179-182, 183-188, 189-195, 196-198, 199-201, 202-205
Methanol	100%	206-223

TLC is a simple, rapid, and inexpensive procedure that gives a quick answer as to how many components are in a mixture. From the TLC analysis 202-205 showed clear single spot with same R_f value 0.6cm and 50 mg was yielded from ethyl acetate: methanol (1:9) ratio fraction of *Cayratia trifolia* stem parts, shown in figure 1.

Figure 1. Thin Layer Chromatography of isolated compound of *Cayratia trifolia*

In UV-Visible spectroscopy analysis, the 202-205 fractions show the maximum absorbance at 271nm, so it confirmed that the compound doesn't have double bond and that bond was not weak (Figure 2).

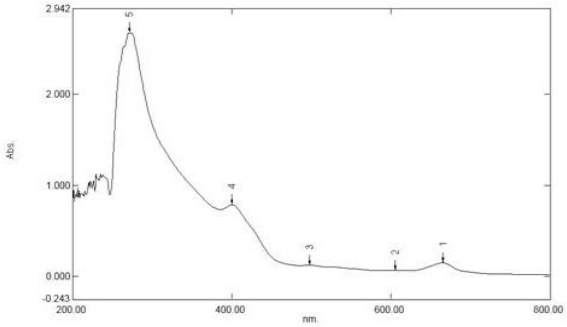

Figure 2. UV Visible Spectroscopy of isolated compound of *Cayratia trifolia*

FTIR confirmed to be a valuable tool for the characterization and identification of compounds or functional groups (chemical bonds) present in an unknown mixture of plants extract. The FTIR analysis showed the presence of O-H at 3442 cm^{-1}, C-H at 2927cm^{-1} and C-O at 1061 cm^{-1}, groups (Figure 3 and Table 2).

Table 2. FTIR spectrum peak values and functional groups of 202-205th fractions

Functional Groups		Type of Vibration	Characteristic Absorptions (cm^{-1})
O-H	Alcohol	Stretch	3442
C-H	Alkane	Stretch	2927
C-O	Alcohol	Stretch	1061

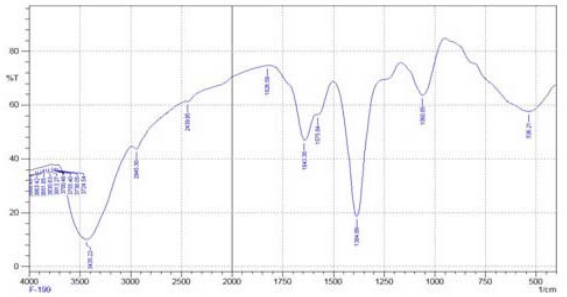

Figure 3: FTIR Spectroscopy of isolated compound of *Cayratia trifolia*

The ^{1}H NMR spectrum showed the presence of a triplet at δ 0.86 for a terminal methyl group, a broad singlet at δ 1.25 showing the presence of a long chain of methylene groups, at δ 2.34 for a methylene α- to the oxy methylene

group, at δ 2.17 for a β – methylene to a oxy methylene group and a pair of multiplet signals at δ 3.6 to a oxy methylene group (OCH_2) (Figure 4).

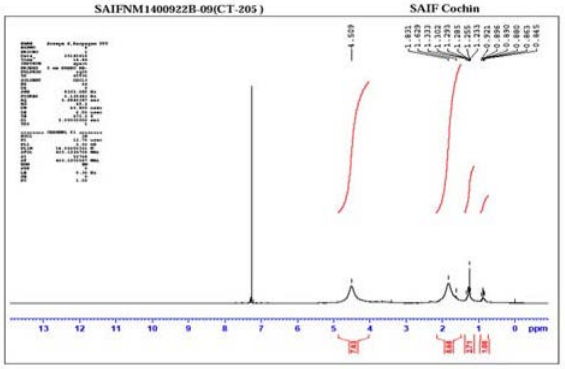

Figure 4. ^{1}H NMR Spectroscopy of isolated compound of *Cayratia trifolia*

^{13}C NMR spectrum indicates the presence of long chain methylene groups (Figure 5). Based on the chromatographic and spectrum techniques indicated that the isolated compound is a 1-pentacosanol (Figure 6 and Table 3).

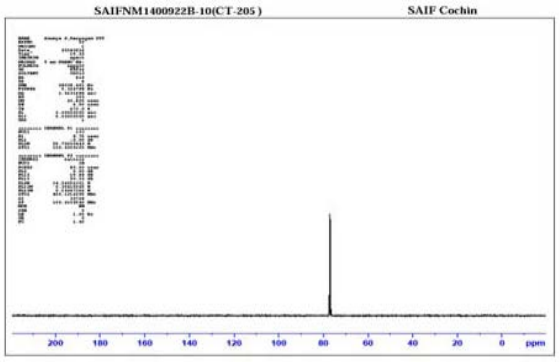

Figure 5. ^{13}C NMR Spectroscopy of isolated compound of *Cayratia trifolia*

Figure 6. Structure of the isolated compound

Table 3. Isolated compound from stem ethanolic extract of *Cayratia trifolia*

Compound Name	1-pentacosanol
Molecular Formula	$C_{25}H_{52}O$
Molecular Weight	368.68 g/mol
IUPAC Name	Pentacosan-1-ol

IUPAC=International Union of Pure and Applied Chemistry

3.1. Molecular Docking analysis

The isolated natural compound was docked with targeted proteins such as AKT and E2F3, PTEN and SMO using Glide module from Schrodinger suite. Based on glide score and glide energy, the docking results were analysed. The docking result of the isolated 1-pentacosanol compound was complex, and the interaction of amino acids with AKT, E2F3, PTEN and SMO protein is shown in table 4.

Table 4. Docking Results of isolated natural compound and standard drug complexed with AKT, E2F3, PTEN and SMO proteins

Target protein	Amino Acids interaction		Ligand atom		Glide Gscore/ Glide energy	
	Finasteride	Pentacosanol	Finasteride	Pentacosanol	Finasteride	Pentacosanol
AKT	ALA 230 (H)	LYS 276(H)	O	O	-3.1/-22.168	-3.42/-34.919
		LYS 179(H)		O		
		ASP 292(C)		H		
E2F3	SER 147 (O)	GLN 303(O)	O	H	-3.8/-41.588	-3.57/-36.846
		ARG 121(O)		H		
PTEN	LYS 330 (H)	ASP 153(O)	O	H	-3.1/-40.050	-3.96/-39.27
		ARG 172(H)	H	O		
SMO	No Interaction	ILE 413 (O)	No Interaction	H	No Interaction	-3.98//-31.761

The molecular docking results revealed that AKT (Fig. 7), E2F3 (Fig. 8), PTEN (Fig. 9) and SMO (Fig. 10) complex with 1-pentacosanol has good glide score of -3.428, -3.573, -3.964 and -3.987 Kcal/mol and the glide energy is -36.846, -31.761, -39.270 and -34.919 Kcal/mol respectively. When compared with standard drug, i.e., Finasteride (complex with AKT, E2F3, PTEN and SMO (no interaction) has low glide score and glide energy -3.1/22.168, -3.8/-41.588 and -3.1/-40.050 Kcal/mol respectively. An ADME property of the isolated natural compound of 1-pentacosanol is shown in Table 5 and was under acceptable range. Perumal *et al.,* (2016) reported that the bioactive compound, epifriedelanol isolated from the ethanolic extract of *Cayratia trifolia* having binding affinities against few proteins (HER2, EGFR and CXCR4) might act as good inhibitor against ovarian cancer.

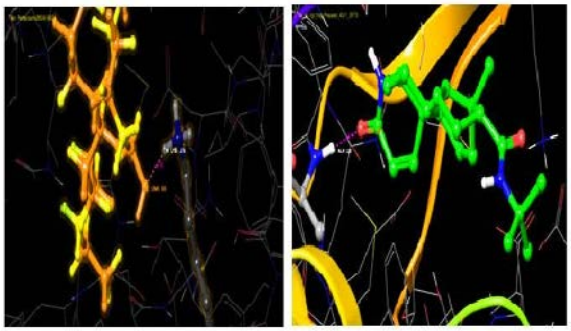

Figure 7: The 3D structure of 1-pentacosanol and finasteride complexed with AKT protein7: (a) 1-pentacosanol 7: (b) Finasteride

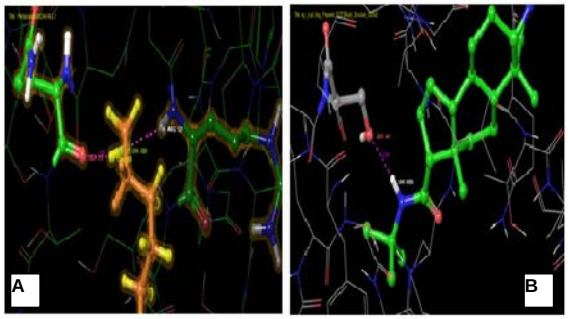

Figure 8. The 3D structure of 1-pentacosanol and finasteride complexed with E2F3 protein 8: (a) 1-pentacosanol 8: (b) Finasteride

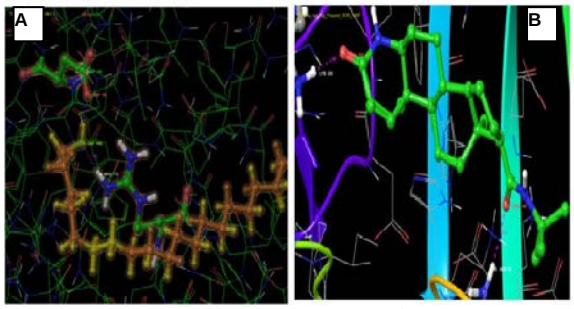

Figure 9. 3D structure of 1-pentacosanol and finasteride complexed with PTEN protein 9: (a) 1-pentacosanol 9: (b) Finasteride

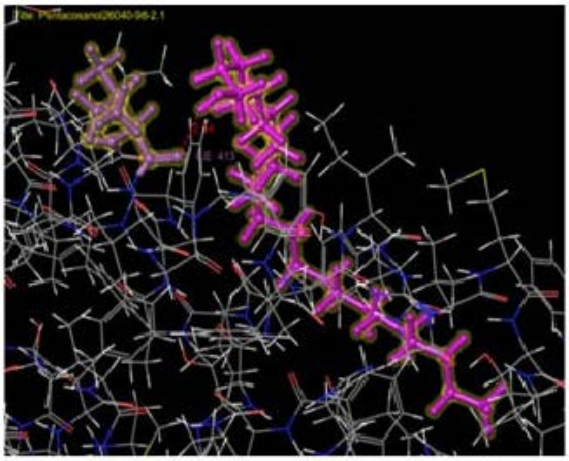

Figure 10. 3D structure of 1-pentacosanol complexed with SMO

Table 5.ADME properties of 1-pentacosanol

Ligand	Molecular weight (g/mol)	H-Bond donar	H-Bond acceptor	Log P (O/W)
1-pentacosanol	368.68 g/mol	1	1	12.2

ADME=Absorption, Distribution, Metabolism and Excretion

4. Discussion

Nature has been a source of medicinal agent for thousands of years and an impressive number of modern drugs have been isolated from natural sources (Nair *et al.*, 2005). Natural products discovered from medicinal plants have played an important role in combating cancer irrespective of their multifactorial origin (Rajkumar *et al.*, 2012). Isolation of pharmacologically active compounds from medicinal plants persist today. Investigation of the chemical composition and secondary metabolites from medicinal plants is an active research field and is the base for drug discovery, due to the demand for identifying and analysing the target proteins with their active sites and potential drug molecules that can bind to these sites specifically.

Cayratia trifolia (Vitaceae), known as fox grape in English, a perennial climber having trifoliate leaves (2–3 cm), is native to India, Asia and Australia. The methanolic extract is more effective than aqueous extract of *Cayartia trifolia* were found to be defending against esophageal cancer in rodents (Rejitha and Das 2009). *Cayratia trifolia* (L.) leaves contain stilbenes such as piceid, reveratrol, viniferin and ampelopsin. Stem, leaves and roots are reported to possess hydrocyanic acid and delphinidin. The leaves were used to cure swelling, injury and infection for bullock (Kumar et al., 2011). Sowmya et al (2016), also reported that more phytochemical and bioactive compounds were present in the stem ethanolic extract of *Cayratia trifolia (L.)* confirmed by FTIR, HPTLC and GC-MS analysis. The ethanolic root extract of *Cayratia trifolia* having antidiabetic activity was reported by Mohammed et al, (2017).

Many databases are available today to describe the medicinal plants and compound (Nonita *et al.*, 2012). The current study provides useful insights to research in isolation and identification of potential anticancer chemo preventive metabolites from stem parts of ethanolic extract of *Cayratia trifolia*. The identification of natural compounds using chromatography and spectroscopic techniques may provide efficient information concerning qualitative and quantitative composition of herbal medicines (Barbosa *et al.*, 2013). Docking is a method which predicts the preferred direction of one molecule to a second when bound to each other to form a stable complex (Tripathi *et al.*, 2012).

The target of ligand-protein docking is to predict the predominant binding model of a ligand with a protein of known three-dimensional structure (Srivastava *et al.*, 2010). The highest negative value of glide score and glide energy indicated that these complexes may have good affinity (Srinivasan *et al.*, 2014). In the present study, the isolated natural compound 1-pentacosanol has comparable good affinity with selected prostate cancer targets of PTEN, AKT, SMO and E2F3 when compared with FDA approved drug of Finasteride.

5. Conclusion

Based on the results, it can be concluded that the isolated bioactive compound of 1-pentacosanol may act as a good inhibitor to the selected targets and a novel anti-prostate cancer agent in future. However, the molecular docking studies alone cannot completely support to control the prostate cancer. The combined *in silico* approach has been translated into *in vitro* and *in vivo* molecular studies which have provided encouraging *in silico* results against the prostate cancer.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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