ORIGINAL ARTICLE

Anticariogenic Activity of Steroid Phytocompound Isolated from *Acmella calva* (DC.) R.K. Jansen

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Abstract

Aim: The phytocompound Stigmast – 5 – En - 3 Beta – Ol isolated and characterized through spectral studies was analyzed for its anticariogenic activity through disc diffusion method and *in silico* analysis. **Materials and Methods:** Anticariogenic activity was carried against dental caries causing microbes such as *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus sobrinus,* and *Staphylococcus aureus* at different concentration (20, 40, 60, 80, 100,120, 140, and 160 as robes such as Autonomous), Affiliated to Bharathidasan University, Tiruchirappalli against *S. mutans*. In *in silico* analysis, the molecular docking of phytocompound was analyzed against eight proteins of four pathogens which were retrieved from PDB database. **Results and Discussion:** The phytocompound Stigmast – 5 – En - 3 Beta – Ol showed good response against all proteins. Among these, the highest ligand and protein interaction was noticed in 3AIE protein of *S. mutans*. The steroid compound was tested for its drug likeness and absorption, distribution, excertion, metabolism, and toxicology properties. The results revealed that the compound accepted the three rule out of four in Lipinski rule of five. ADME properties and toxicity of the phytocompound showed oral bioavailability which are under permissible limit. **Conclusion:** From the present investigation, it was proved that the phytocompound Stigmast – 5 – En - 3 Beta – Ol is harmless and effect potent against dental caries causing microbes.

Key words: Bioavailability, drug likeness, interaction, ligand, Stigmast – 5 – en - 3 beta – Ol, toxicity

INTRODUCTION

Infectious diseases are the main reason for causing death all over the world. Resistance to antibiotics are the major concern across the global.[1] Because of the emergence of the multidrug-resistant pathogens, the potency of many subsist antibiotics is being threatened.[2]

Pain is the important thing that brings the patient to medicinal practitioner. Tooth decay and periodontal disease are the common dental infectious problems caused by bacteria in humans. These infections are non-life-threatening in nature and their existence have minimized their importance in human health. Dental caries is a transmissible, localized, and pathological infectious method that may lead to destruction of the structures of teeth.[3] The public health problem are major in India responsible for the prevalence of dental caries in children is high as 60–80%.[4]

In recent years, natural medicines have multiple pharmacological components when compared with chemical drugs in aspect of low toxicity, multiple targets, a wide range of sources which received greater attention.[5] Hence, they are increasingly used as alternatives to chemical drugs. Investigation of the chemical components of the different plants and their pharmacological screening may give us the basis for the development of novel agents. In addition, medicinal herbs have blessed us with essential lifesaving drugs used as an equipment for modern medicine. Among 400,000 plant species, only 15% have been phytochemically studied and 6% of plant species are investigated for its biological activity.[6] This clearly shows the evaluation of the herbal drugs activity according to

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their phytopharmacological compounds. According to the World Health Organization survey 80% of the populations depended upon the traditional herbal medicine to seek the primary healthcare.[7]

Computational biology and bioinformatics have the potency for speeding up the drug discovery process by reducing the cost and also changing the route how the drugs are designed. Rational drug design helps to facilitate the designing process and involves variety of methods to recognize novel compounds. Docking is one of the method which involves the interaction of the drug molecule (Ligand) with the receptor (Target). To identify the action antimicrobial active compounds *in- silico* molecular docking studies were involved. The computational tools are used to understand the structural properties of the metabolites and antibacterial actions.[8]

In the present study, *in silico* studies were done for the metabolites of the plant *Acmella calva* (*DC*.) *R.K. Jansen* against *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus sobrinus,* and *Staphylococcus aureus*. The molecular docking result would help in the identification of the bioactive compound responsible for antimicrobial activity.

MATERIALS AND METHODS

Collection and authentication of the plant material

A. calva (DC.) R.K. Jansen whole plant was collected from the Nanjikottai road, Tanjore, Tamil Nadu, India, in the month of October. The whole plant was washed and shade dried under room temperature. The dried plant was powdered coarsely. The plant was identified and authenticated by Dr. S. Soosairaj, Assiatant Professor, St. Joseph's College, Tiruchirappalli – 02 in accordance with the "Flora of Central and Northern Tamil Nadu" by John Britto S (2019) and the specimen accessed as 3002.

Preparation of ethanol extracts

The ethanol extracts were prepared by soaking 400 g of the dried powder plant materials in 2 L of ethanol by using a soxhlet extractor for 10 h continuously. The extracts were filtered through Whatmann filter paper No. 42 (125 mm). The filtered extract was concentrated and dried by using a rotary evaporator under reduced pressure. The final condensed dried samples (340 g) were stored in labeled sterile bottles and kept at −20°C.

Isolation of bioactive compounds by column chromatography

The condensed ethanol extract of whole plant (340 g) of sample was subjected to column chromatography over TLC grade silica gel. The column was eluted with n-hexane, then with ethyl acetate in n-hexane and finally with methanol yielded a number of fractions. The preparation of solvent systems used to obtain white powdered compound (143 mg/340 g) ethyl acetate: Methanol (50:50v\v) from fractions 25. The compounds were detected on TLC plates by spraying with Libermann Burchard reagent and heated at 100°C for 10 min.

Collection of test pathogens

The antibacterial activity of isolated compounds was exhibited against *S. mutans* (microbial type culture and collection [MTCC] 890), *S. salivarius* (MTCC 13429), *S. sobrinus* (MTCC 33479) and *S. aureus* (MTCC 25923) were prepared as test organisms. All the bacterial strains were purchased from the MTCC at Chandigarh, India.

Determination of antibacterial activity by disc diffusion method

The disc diffusion method is used to evaluate the antibacterial activity of each isolated compounds. The isolated compounds (10 mg) were redissolved in 1 mL of ethanol, sterilized through Millipore filter (0.22 µm) then loaded over sterile filter paper disc (8 mm in diameter to obtain final concentration of 10 mg/disc. Ten mL of Mueller-Hilton agar medium was poured into sterile petri dishes (diameter 60 mm) and inoculated with test organism. Sterile filter paper disc loaded with various concentrations of isolated compounds of 20, 40, 60, 80, 100, 120, 140, and 160 mg/mL were placed on the top of Mueller-Hilton agar plates. Filter paper disc loaded with 5 µg of amoxicillin was used as positive control. Negative control was prepared using the respective solvent. The plates were incubated at 37°C for 24 h and the zone of inhibition was recorded in millimeter and the experiment was repeated twice.

Chemsketch

The structure of the compound Stigmast – 5 – En - 3 Beta – Ol one was drawn using an integrated software package called Chemsketch which was organizing by Advanced Chemistry Development Inc. for drawing chemical structure. The 2D model was converted to 3D model [Figure 1].

Lipinski rule of five and absorption, distribution, excertion, metabolism, and toxicology (ADME/T) properties

Lipinski rule of five and ADME/T properties was calculated using the link http://www.swissadme.ch/for the approval of phytochemical compound as drug. ADME/T Properties is an online based program for building drug-like library and predicting ADME data using *in silico* method.

Molecular docking

Ligand preparation

3D models of Stigmast – 5 – En - 3 Beta – Ol were further optimized and prepared by Autodock tool.

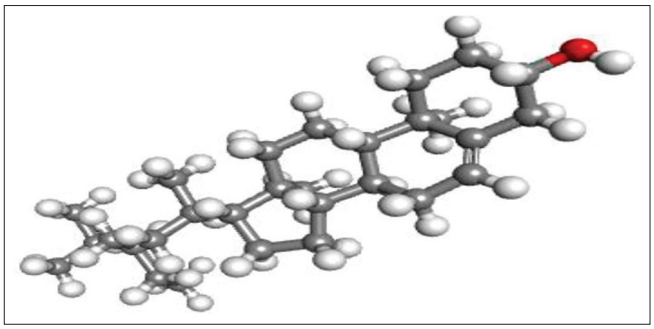

Figure 1: Structure of Stigmast – 5 – en- 3 beta- ol

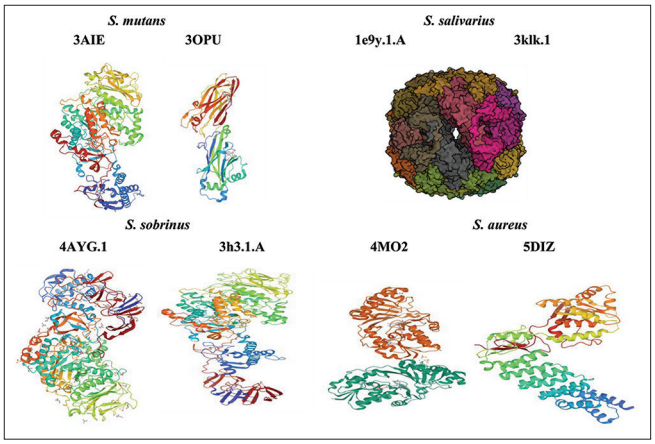

Figure 2: Proteins of Dental microbes

Protein preparation

The target proteins of *S. mutans* (3AIE and 3OPU), *S. salivarius* (1e9y.1.A and 3klk.1), *S. sobrinus* (4AYG.1 and 3hz3.1.A), *and S. aureus* (4MO2 and 5DIZ) was retrieved form PDB database [Figure 2]. The RCSB PDB (http://www.rcsb.org) also provides a variety of tools and resources about the experimentally determined structures of proteins, nucleic acids, and complex assemblies the molecules are visualized, downloaded, and analyzed for further studies. The protein was lead to energy minimization (CHARMM force field). The active site of the protein was first identified by Castp 3.0 which is used to define the binding site.

Docking

Molecular docking (*in-silico* docking) is a method which identify the correct conformations of one molecule to a second one when bound to each other to form a stable complex. The docking study of Stigmast – 5 – En - 3 Beta – Ol one with two proteins of all four microbes was carried out by Ligand Fit of Discovery studio (Version 2.1, Accelry's Software Inc.). It predicts the strongest binders based on its scoring functions and software allows to virtually screen the database of a compounds. It explores the ways in which the molecules and its receptors fit together and dock to each other well. The ligand was docked with eight proteins of four microbes and the results were analyzed.

RESULTS AND DISCUSSION

Identification of the compound

The phytocompound constitutes in the whole plant extract of rare medicinal herb *A. calva* (*DC.*) *R.K. Jansen* was reported

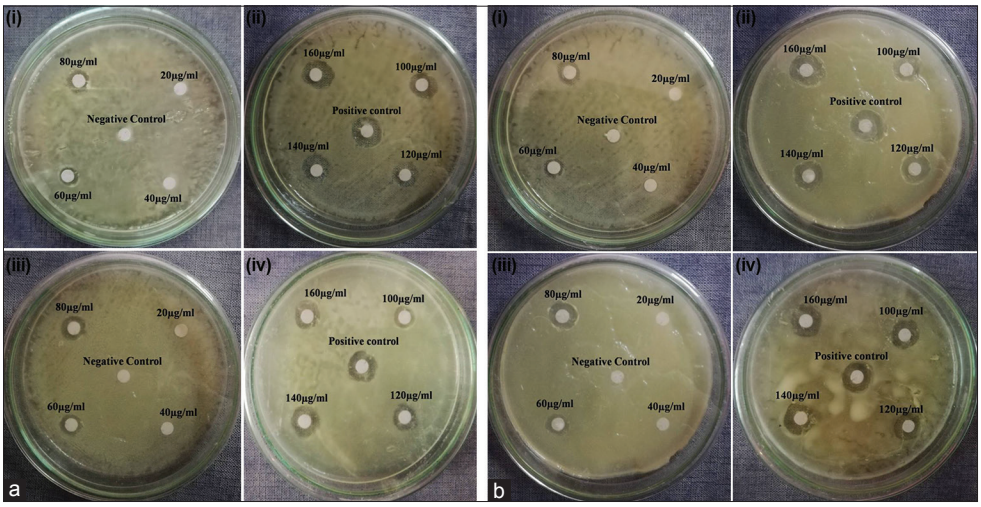

Figure 3: (a) Anticariogenic activity of the isolated compound Stigmast – 5 – en – beta – ol from the ethanolic extract of *Acmella calva* (DC.) R. K. Jansen against *Streptococcus mutans and Streptococcuss salivarius.* (a-i and a-ii) – Effect of Stigmast – 5 – en – beta – ol on *Streptococcus mutans.* (a-iii and a-iv) – Effect of Stigmast – 5 – en – beta – ol on *Streptococcus salivariu.* (b) Anticariogenic activity of the isolated compound Stigmast – 5 – en – beta – ol from the ethanolic extract of *Acmella calva* (DC.) R. K. Jansen against *Streptococcus sorbinus and Staphylococcus aureus.* (b-i and b-ii) – Effect of Stigmast – 5 – en – beta – ol on *Streptococcus sorbinus.* (b-iii and b-iv) – Effect of Stigmast – 5 – en – beta – ol on *Staphylococcus aureus*

Table 1: Anticariogenic activity of the compound Stigmast–5–en–3 beta–ol isolated from ethanolic extract of *Acmella calva* (DC) R. K. Jansen against four dental caries causing organisms

Isolated compounds	Concentration (µg/mL)	Organisms/zone of inhibition (mm)			
		Streptococcus mutans	***Streptococcus salivarius***	***Streptococcus sobrinus***	***Staphylococcus aureus***
Stigmast–5–en–3 beta–ol	20	0.8±0.2	0.7±0.76	0.7±0.2	0.66±0.5
	40	0.3±0.57	0.8±0.34	3.8±0.2	2.06±0.50
	60	4.9±1.01	5.96±0.5	5.6±1.04	4.5±1.80
	80	4.5±1.5	6.08±0.94	7.08±0.3	6.03±0.3
	100	8.4±0.6	6.1±0.9	7.7±0.6	8.1±0.36
	120	6.8±2.02	6.7±0.3	6.3±0.60	8.03±0.20
	140	9.2±1.10	9.03±0.41	8.23±0.50	8.23±0.25
	160	10.2±0.04	8.4±0.75	8.46±0.59	8.73±1.10
	Negative control (ethanol)	0	0	0	0
	Standard (amoxicillin) positive control	8.7±0.86	8.7±0.5	7.66±0.95	8.6±0.55

Values are mean±SE of three experiments. SE: Standard error

Table 2: Lipinski rule of five of Stigmast–5–en–3 beta–ol

Serial number	Lipinski rule of five	Stigmast–5–en–3 beta–ol
1.	Molecular weight	414.7 g/moL
2.	Hydrogen bond donor	1
3.	Hydrogen bond acceptor	1
4.	Logp	20.23*

Table 3: ADME/T properties of Stigmast–5–en–3 beta–ol

Serial number	ADME/T properties	Stigmast–5–en–3 beta–ol
1.	BBB	–0.024
2.	PPB	100
3.	Skin permeability	–2.20
4.	HIA	98%
6.	$CaCO_2$	38.6
7.	Bioavailability	0.55

HIA: Human intestinal absorption, BBB: Blood brain barrier, PPB: Plasma protein binding

as Stigmast – 5 – En - 3 Beta – Ol through NMR spectral studies in our previous investigations.[9]

Anticariogenic activity

The anticariogenic activity of the isolated phytocompound of Stigmast – 5 – en - 3 – beta – ol of *A. calva* was studied in different concentrations (20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL, 100 µg/mL, 120 µg/mL, 140 µg/mL, and 160 µg/mL) against dental caries causing pathogens such as, *S. mutans, S. salivarius, S. Sobrinus,* and *S. aureus*. Almost all the concentration showed antibacterial activity against all four microbes (*S. mutans*, *S. salivarius*, *S. sobrinus,* and *S. aureus*). The data pertaining to the

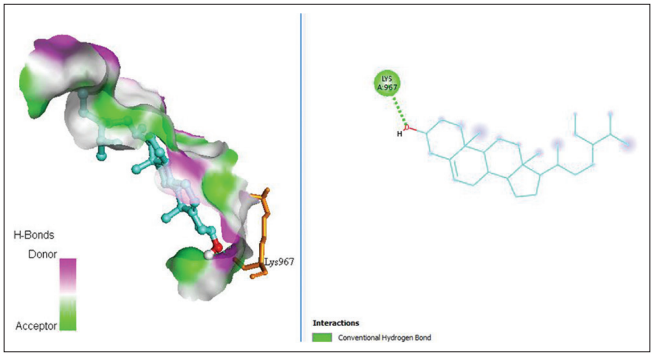

Figure 4: The hydrogen bonded interaction of 3AIE protein of *Staphylococcus mutans* with Stigmast – 5 – en – beta – ol

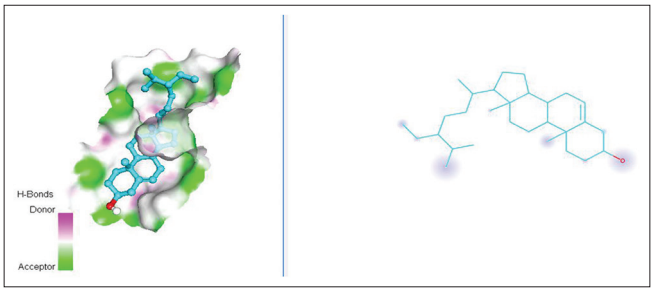

Figure 5: The hydrogen bonded interaction of 3OPU protein of *Staphylococcus mutans* with Stigmast – 5 – en – beta – ol

Table 4: Molecular docking studies of Stigmast–5–en–3 beta–ol against 8 proteins of dental caries causing microbes

Compound	Microbes	Proteins	Number of poses	Absolute energy	Libdock score	H-bond	Residue	Bond length
Stigmast – 5 – en- 3 beta- ol	*Streptococcus mutans*	3AIE	205	7.52	85.41	1	Leucine 967	2.01
		3OPU	262	-	-	-	-	-
	Streptococcus salivarius	1e9Y.1.A	127	7.49	32.16	1	Methionine 77	1.91
		3klk. 1	160	8.92	76.36	1	Leucine 774	2.0
	Streptococcus sobrinus	4Ayg. 1	295	9.69	78.97	1	Aspirin 286	1.92
		3hz3.1.A	161	7.22	51.34	1	Tryptophan 187	2.15
	Staphylococcus aureus	4MO[2]	83	7.0	73.52	1	Tyrosine 620 Serine 622	2.38 1.74
		5DIZ	115	8.05	71.5	1	Glycine 121	1.98

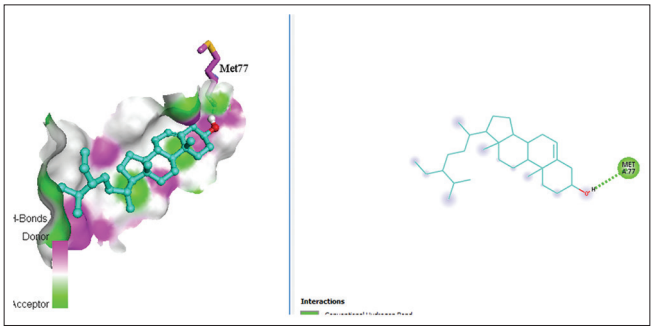

Figure 6: The hydrogen bonded interaction of 1e9y.1.A protein of *Staphylococcus salivairus* with Stigmast – 5 – en – beta – ol

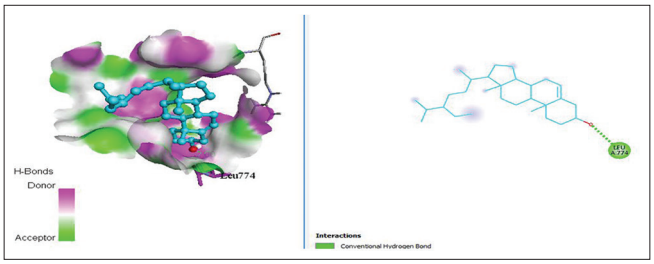

Figure 7: The hydrogen bond interaction of 3klk.1 protein of *Staphylococcus salivairus* with Stigmast – 5 – en – beta – ol

antimicrobial potency of the phytocompound Stigmast – 5 – en - 3 – beta – ol are present in Table 1. The activity was determined by measuring the "zone of inhibition" around the disc.

In the concentration of 160 µg/mL, 140 µg/mL, 120 µg/mL, and 100 µg/mL, the maximum range of 11 to 8 mm zone of inhibition were noted and in 80 µg/mL and 60 µg/mL concentration 7–2 mm were recorded [Figure 3a and b]. The lower concentrations (40–20 µg/mL) showed poor response against all four bacteria's. Similar results were reported with the crude extracts of different plants containing steroid compounds against various microorganisms.

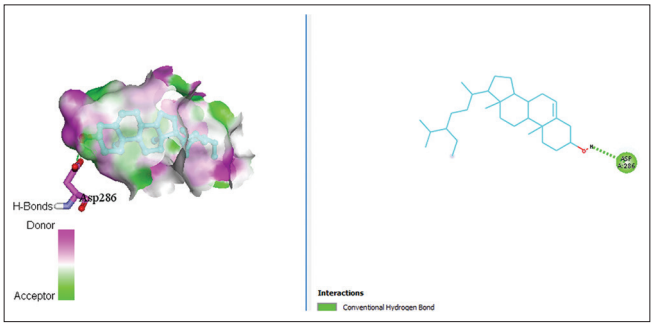

Figure 8: The hydrogen bond interaction of 4AYG.1 protein of *Staphylococcus sobrinus* with Stigmast – 5 – en – beta – ol

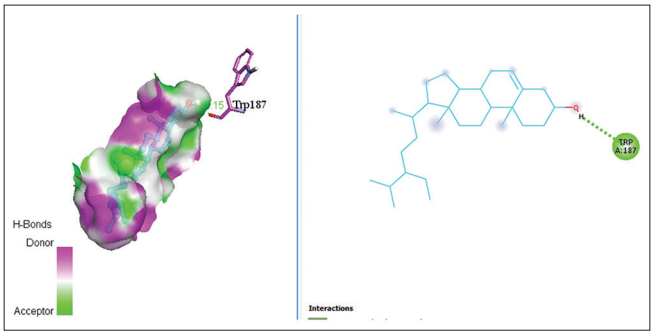

Figure 9: The hydrogen bond interaction of 3hz3.1.A protein of *Staphylococcus sobrinus* with Stigmast – 5 – en – beta – ol

Namwase *et al*., 2021[10] evaluated the antibacterial activity of aqueous extract of *Corchorus olitorius* L. and ether extract of *Acmella caulirhiza* Del. against *S. mutans* has the highest zone of inhibition at the concentration of 1000 mg/mL and lowest MIC and MBC values.

Syahirah *et al*., 2020[11] investigated the antibacterial activities of *Acmella paniculata* leaves and flowers extract against *S. mutans* using disc diffusion, MIC, and MBC methods. Crytal violet assay was used to find out the anti-biofilm activity of all the extracts. They found out that n-hexane and methanol extract from leaves showed the

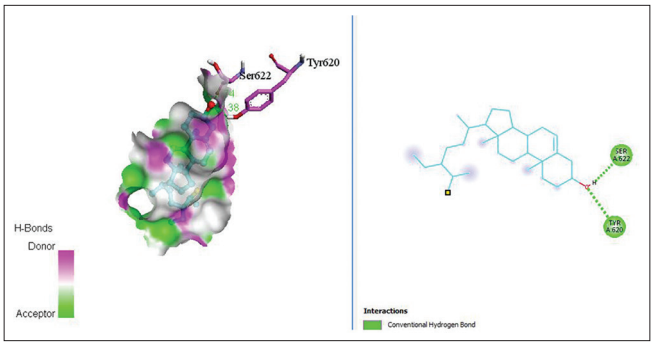

Figure 10: The hydrogen bond interaction of 4MO2 protein of *Staphylococcus aureus* with Stigmast – 5 – en – beta – ol

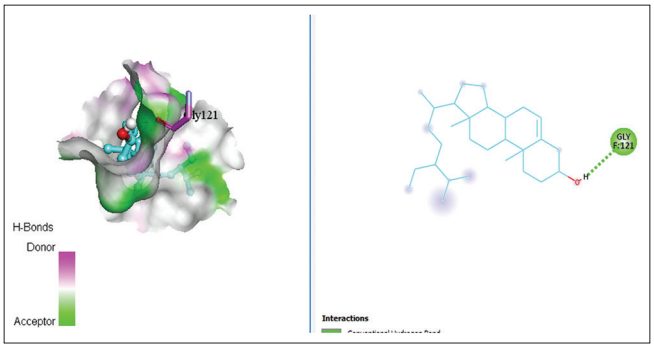

Figure 11: The hydrogen bond interaction of 5DIZ protein of *Staphylococcus aureus* with Stigmast – 5 – en – beta – ol

highest inhibition one against *S. mutans* compared to DCM and acetone extract. The MBC values were 50–100 mg/mL and MIC were 25 mg/mL. They concluded that n-hexane leaves extract, methanol leaves extract, n-hexane flowers extract, and DCM flowers extract of *A. paniculata* showed bactericidal properties against *S. mutans*.

The structure of Stigmast – 5 – En - 3 Beta – Ol was drawn using various tools in Chemsketch and properties were predicted [Figure 4]. The structure of Stigmast – 5 – En - 3 Beta – Ol has 21 carbons, 34 hydrogens and 1 oxygen atom in its structure. The Lipinski Rule of Five is calculated to see drug likeness property of the compound Stigmast – 5 – En - 3 Beta – Ol and given in the Table 2. The Lipinski Rule of Five gives the molecular weight, hydrogen bond donor, hydrogen bond acceptor, and Log p value which are 414.71 g/mole, 1, 1, 20.23, respectively, for the compound Stigmast – 5 – En - 3 Beta – Ol. The compound accepted three rules out of four and hence, the compound could be used as a potent drug.

Alodeani *et al*.,[12] evaluated the alkaloids in black pepper for its drug likeness and physicochemical properties using an online software called Molinspiration. The computational studies revealed that all alkaloid except piperidine were found to possess good bioactivity score. It indicates that only the combination of all three functionality was responsible for good biological activity.

The ADME/T properties such as blood brain barrier (BBB), plasma protein binding (PPB), skin permeability, human intestinal absorption (% HIA), Caco-2 cell model, and bioavailability score were predicted and given in Table 3. The result was −0.024 BBB, 100 PBP, −2.20 skin permeability, 98% HIA, 38.6 CaCo2, and 0.55 bioavailability.

An almost identical result was given by Daisy and Kani 2013[13] were they studied the Biotin, a key enzyme participates in indespensible of bacterial fatty acid for the existence of *Mycobacterium tuberculosis*. Target protein from *Mycobacterium* was affected its fatty acid synthesis leads to death of the organism. Molecular docking study on the enzyme from *Gloriosa superb*. Drug like properties of these ligand was calculated by ADME calculations. Based on the molecular docking results and ADME values, *Gloriosal* was confirmed as a promising lead compound.

ADME/T predicts BBB model for Stigmast – 5 – En - 3 Beta – Ol and BBB shows negative value −173.760 and clearly predict that it is CNS inactive compound and has no capacity to cross BBB. PPB of the phytochemical compound Stigmast – 5 – En - 3 Beta – Ol one shows 100% bound-ness which is the efficacy as one the quality of drug.

In molecular docking, the active sites of eight modeled proteins of four microbes were predicted and identified. The hydrogen bonds of eight proteins were found out and these hydrogen bond formations contribute in the interaction of ligand and protein. There were two hydrogen bonds in 3AIE protein and one hydrogen bonds in 3OPU protein of *S. mutans*. The ligand protein interaction details are given in Table 4. Stigmast – 5 – En - 3 Beta – Ol interacted with 3AIE protein in lysine 967 with the libdock score of 83.16 with a distance of 1.77 [Figure 4] and with 3OPU protein no hydrogen bond interactions were found [Figure 5].

Stigmast – 5 – En - 3 Beta – Ol interacted with 1e9y.1.A protein with one hydrogens (methionine 177 with a distance of 1.91) having a libdock score of 76.7 [Figure 6] and 3klk.1 protein with one hydrogens (Leucine 774 with a distance of 2.00) having a lib dock score 74.32 [Figure 7] of *S. salivarius*. The isolated phytocompound interacted with one hydrogen of 4AYG.1 (Aspirin 286) with a distance 1.96 and libdock score of 69.56 and 3hz3.1. A (tyrosine 187) with a distance 1.92 and libdock score of 73.09 proteins of *S. sobrinus* [Figures 8 and 9]. Two hydrogens and one hydrogen from each protein of *S. aureus* interacted with Stigmast – 5 – en – 3 beta – ol [Figures 10 and 11] with a lib dock score of 70.3 (Serine 622) with a distance of 2.15 and 1.74. And 5DIZ protein interacted with 66.8 (Glycine 121) with a libdock score of 82.78 with a shorter distance of 1.98. These interactions depicted that, the ligand (Stigmast – 5 – En - 3 Beta – Ol) fit exactly in the active sites of eight proteins of four microbes. However, the highest receptor-ligand binding affinity was found in proteins of *S. mutans* with Stigmast – 5

– En - 3 Beta – Ol. The absolute energy (the energy spent by protein during interaction) was also calculated. The absolute energy of each proteins was also calculated.

Rao *et al*., 2021[14] investigated three bioactive compounds from *Morus alba*. *C*athafuran-B supported the result of *in silico* analysis showing that it could be a potential glucosamine-6-phosphate synthase inhibitor. Thus, biomolecule exploring its pharmacological properties and lowering the utilization load present on highly explored *M. alba*.

CONCLUSION

The isolated compound Stigmast – 5 – En - 3 Beta – Ol from *A. calva* (DC.) R. K. Jansen was analyzed for its antibacterial action through disc diffusion and docking studies. The phytocompound showed good anticariogenic activity against selected human oral pathogens and proved to be lead compound by satisfying Lipinski rule of five and ADME/T properties. Hence, the compounds proved to be very effective against dental caries causing microbes.

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