ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 16, Issue 12, 2023

Online - 2455-3891
Print - 0974-2441

Research Article

BIOCHEMICAL EVALUATION OF INDUS VIVA I PULSE NATURAL AYURVEDIC SYRUP AND IT'S *IN SILICO* – INTERACTION ANALYSIS

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Received: 29 August 2023, Revised and Accepted: 10 October 2023

ABSTRACT

Objectives: In developing countries like India, diabetes mellitus and human bone cancer are progressively increasing which is global encumbrance. The objective of the study is to find out the phytoconstituents of Indus Viva I Pulse health drink extract that has undergone classical chemical characterization, molecular docking, anti-diabetic, anticancer, antibacterial and antifungal studies used as traditional and complementary medicine. The study aimed to evaluate the Gas Chromatography–Mass Spectrometry (GC-MS), High-Performance Liquid Chromatography (HPLC), Liquid Chromatography–High Resolution Mass Spectrometry (LC-HRMS), anticancer, antidiabetic and spectral studies that are used to disclose phytochemicals and their medicinal properties.

Methods: Infrared (IR), Ultraviolet (UV), ^{1}H-NMR spectroscopic methods, GC-MS, HPLC and LC-HRMS instrumental detections were employed. Molecular docking, anti-diabetic, anticancer, antibacterial and antifungal studies were carried out using AutoDock 4.2.6. software program, α- Amylase method, Mosmann and Disk diffusion method respectively.

Results: GC-MS and HPLC chromatogram of methanol extracts exhibited 12 and 6 peaks respectively, confirming the presence of 12 phytoconstituents in Indus Viva I Pulse health drink (1I–12I). The total phenol and flavonoid content found in the extracted sample of Indus Viva I Pulse health drink were 0.16 and 0.36 mg/mL respectively. It is a polyherbal formulation of brown-colored liquid of the UV spectrum in methanol is characteristic of an aromatic compound with λ max of 319 and 412 nm. IR spectrum gave peaks at 3,457, 2,922, 2,857, 2,121, 1,641, 1,055, 1,033 and 621 cm^{-1} indicating the presence of an alcoholic, alkyl, aldehyde or ketone, alkyne, carbonyl, anhydride and methyl group respectively. Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) study gave these molecular weights (m/z): 1134. 7017, 1150.6475 and 1168.6797. Aromatic characteristics were confirmed through UV, IR, ^{1}H-NMR, ^{13}C-NMR and LC-HRMS spectral studies. ^{1}H-NMR study indicates the presence of aromatic protons and methyl protons. Aqueous extract of Indus Viva I Pulse health drink was tested for Cytotoxicity Assay of human bone cancer (MG-63) cell lines and ptyalin prevent characters that exhibited a valid inhibitory value of 90.87% in 10 µg/mL for MG-63 and value of 74.41% in 500 µg/mL for alpha-amylase which is comparatively more efficient than the available standard drug. Cytotoxicity assay for human bone cancer (MG-63) cell lines and alpha-amylase both showed the inhibitory activity of IC_{50} 68.95 and IC_{50} 36.18 µg/mL respectively.

Conclusion: Based on the result of this research, it can be proposed that the Indus Viva I Pulse health drink may serve as a potential remedy for the human bone cancer (MG-63) cell line and alpha-amylase inhibitory activity is proportional to the dose.

Keywords: Amylase, Osteosarcoma, Microbial, Docking, Ayurvedic and Herbal.

 DOI: http://dx.doi.org/10.22159/ajpcr.2023v16i12.49266. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Cancer is a serious disease that accounts for about 10 million death cases per year worldwide. Approximately, 70% of deaths due to cancer occur in low-income countries and middle-income countries pose an important impact on economics. The total amount expended for cancer in the year 2010 was around 1.16 trillion U.S. dollars. Updates from the International Agency for Research on Cancer and GLOBOCAN recorded a surge in new cancer cases and subsequent mortality estimating about 10 million death cases out of 19.3 million new cancer cases reported in the year 2020 in contrast to the reported death of 7.6 million from an estimated 12.7 million cancer cases in the year 2008. Considering the rate at which it has increased in the years mentioned above, by the year 2040, new cancer cases may rise to about 28.4 million making 17 million people susceptible to eventual death, which is alarming. In case of bone cancer the prevalence was thought to be extremely rare, has now become the leading cause of cancer death and is still rising at an exponential rate in developing countries [1]. Cancer remains an aggressive killer worldwide although the recent trend observes considerable efforts in the discovery and development of new drugs. Moreover, synthetic chemotherapeutic agents in current use attributed to the heavy cost of their development do not meet the set expectations. Therefore, the current scenario necessitates a new approach to combat the drastic rise in cancer incidents in the forthcoming years and demands the formulation of novel anticancer medicines making them affordable to low-income groups at the same time. 5.4 males, 4.0 females, 6.8 blacks, 6.5 Hispanics, and 4.6 whites per million people are affected by osteosarcoma every year [2]. Although we have modern innovative therapeutic approaches such as excision, computed tomography and radiation therapy (RT) which are used in the treatment of bone cancer found in children and adolescents, the osteosarcoma mortality rate has been increasing. In this point of view, potent medicinal treatment needs for osteosarcoma, plant-derived natural products are safe, effective, cheap, easily available possess fewer side effects and have no toxicity as compared to modern drugs and treatments [3]. Several research articles have been published about herbal medicinal plants that cure osteosarcoma owing to the natural chemopreventive properties of polyphenols, flavonoids, terpenes, and alkaloids. Among the identified 3,000 medicinal plants exhibiting anticancerous properties, some natural products yielded from them being in current use for cancer treatment include alkaloids, *Taxus diterpenes*, *Camptotheca* and *Podophyllum lignans* [4]. There are four classes of herbal plant-derived compounds, namely vinca alkaloids,

epipodophyllotoxins, taxanes and camptothecin that have undergone clinical trial testing. Further global utilization of phytological materials for chemotherapy ranges around 50% for Asiatic patients. As there is a long tradition of herbal medicinal plants being used for fighting deadly diseases for centuries, they can be considered as an alternative source for discovering novel drugs that shall tremendously enhance the prospects of curative measures for various diseases. Extensive studies have been carried out to date upon highlighting and proving the efficacy of plant-derived medicines in inhibiting various stages of carcinogenesis and to signify the application of novel herbal plant drugs in oncogenesis therapy alongside preventive measures [5]. The multifariousness of metabolites extracted from medicinal plants is known to research for healing several ailments but there is a lack of pertaining the same. Since this provides bounteous opportunities for unraveling novel effective drugs, investigating the chemical structure of these compounds and their biological assessment becomes imperative to elucidate and confirm the potential of the medicine against the targeted disease [6]. On the other hand, Diabetes is an incurable and prolonged disease that can lead to myocardial infarctions, apoplexy, renal disease, trauma, visual impairment and neurological disorders. Diabetic persons rose from 108 million to 422 million between 1980 and 2014, a 5% increase was seen between 2000 and 2016 and 1.5 million diabetic patients deaths died in 2019 [7]. Diabetes could become the seventh leading cause of death in the world by 2030. Biguanides, thiazolidinediones, sulfonylureas, glinides, α-glucosidase inhibitors, dipeptidyl peptidase-IV and SGLT2 are being proven to be better remedies for type 2 diabetes mellitus [8]. Development of gliflozins as SGLT2 inhibitors are dapagliflozin, canagliflozin, iparagliflozin, empagliflozin, luseogliflozin and tofogliflozin. Second-generation sulfonyl urea derivatives approved for antidiabetic activity are glipizide, glimepiride, rapagonide, tolbutamide, chloropropamide, tolozamide and acetohexamide. Biguanides, namely metformin, buformin, and phenoformin medicines are used to treat diabetes [9]. Indus Viva I Pulse health drink is a mixing of herbs and antioxidant fruit blend which contains Acai Berry (*Euterpe oleracea*), Green and black Concord Grapes (*Vitis vinifera*), Pineapple (*Ananas comosus*), Apple (*Pyrus malus*), Bananas (*Musa sapientum*), Oranges (*Citrus aurantium*), Pear (*Pyrus communis*), Pomegranate (*Punica granatum*), Blueberries (*Vaccinium corymbosum*), Blackberries (*Rubus fruticosus*), Strawberries (*Fragaria ananassa*) and Mulberries (*Morus alba*) is used to help management of healthy cholesterol, healthy digestion process, delay the aging process, enhance the energy level, cardiovascular health, anticancer support, respiratory wellness, Cellular wellness, hepatic wellness, vascular wellness, cerebral wellness, muscular skeletal wellness and boosting immunity. According to research studies, the above-mentioned fruit blend is the most powerful immune booster for our body. Considering the premise purported above the following studies, namely Infrared (IR), Ultraviolet (UV), ^{1}H-NMR, ^{13}C-NMR, Liquid Chromatography–High Resolution Mass Spectrometry (LC-HRMS), High Performance Liquid Chromatography (HPLC), Gas Chromatography–Mass Spectrometry (GC-MS), cancer, and diabetics were conducted to prove the efficacy of herbal medicine Indus Viva I Pulse health drink in stabilizing itself as a potent remedy for cancer as well as diabetes.

METHODS

Preparation of Indus Viva I Pulse extract

Indus Viva I Pulse health drink contains 15 exotic fruits that are suitable to be kept in a cool and dry place. Also, it should be stored inside a refrigerator before and after opening the seal. Remember to shake the bottle well before consuming. The Indus Viva I Pulse health drink is extracted at 85–98°C for a period of 15–30 s. The derived liquid was drained through Whatman filter paper 42 and decocted. A light brown color solution was obtained which was further analyzed (or) the freeze/sublimation concentration step can be accomplished by freeze concentration or by sublimation concentration [10].

Estimation of major phytocompounds

Total flavonoid and phenolic content were determined quantitatively following standard procedure. HPLC and GC-MS analysis of Indus Viva I Pulse health drink extracts were carried out by Waters 2545 Quaternary Gradient Module with 2998 Photodiode Array Detector, Vienna, Austria and Agilent 8890-version: 2021-0709-2206-17619 (GC-model) equipped with HP 5 MS column, respectively. HPLC and GC-MS system was applied to identify the chemical components. ^{1}H NMR spectra of the samples were recorded on 400 MHz Bruker using DMSO-d_6 with TMS as the internal standard. LC-MS result of Indus Viva I Pulse health drink extract was done by SAIF, VIT University, Vellore-632014, Tamil Nadu. LC-HRMS of crude Indus Viva I Pulse health drink was recorded on the WATERS–XEVO G2-XS-QToF high-resolution mass spectrometer (LC-HRMS/MS +ve mode) system. UV and IR of Indus Viva I Pulse health drink extract were carried out in the range between 254 and 365 nm and 4400 and 400 cm^{-1}, respectively. All solvents used were of analytical grade.

Docking analysis

The 3D structures of 3BC9 diabetic protein and the structure of PD-1 checkpoint bone cancer protein were obtained from the RCSB PDB database (http://www.rcsb.org) (http://www.rcsb.org/). Indus Viva I Pulse health drink 12 phytoconstituents put through molecular docking with 3BC9 diabetic protein and structure of PD-1 checkpoint bone cancer protein by AutoDock 4.2.6. Software program. This confirmed that actual binding interactions without any prior knowledge of best conformers were imaged with the lowest binding energy (–kcal/moL) revealing information that has kept the pharmacological role of these phytoconstituents. Framework and docking procedures of the compound one by one were performed using Auto Dock Vina. The best-configured pose of the molecule towards 3BC9 and PD-1 checkpoint bone cancer protein from the result of Autodock was taken to further studies [11].

Antimicrobial activity

The antimicrobial activities of Indus Viva I Pulse health drink extracts by the Disk diffusion method were gram stain tested on *Staphylococcus aureus*-902, *Escherichia coli*-443 and fungi namely Candida albicans and Aspergillus niger respectively. The experiment was carried out at the Research Center for Biotechnology, Thillai Nagar (East), Trichy-620 018. The medium was prepared with yeast extract (5 g), meat extract (10 g), peptone (5 g), sodium chloride (5 g) and agar (20 g) and the pH was maintained at 7. The medium was boiled, sterilized and autoclaved at 7 kg pressure (121°C) for 15 min. 20 mL of the media was poured into the sterilized Petri plates succeeding sterilization. For the solidification of media, these Petri plates were kept at room temperature for a few minutes. Microorganisms were inoculated in the medium using simile swabs after the incubation period of 18 h. The entire procedure was carried out under aseptic conditions. Since active plant extracts with potent antimicrobial activity do not change color, the color change (extract color to red) in each well was examined to identify and distinguish them from their counterparts after 30 min [12].

Assessed *in vitro* anti-diabetic activities

The α- α-amylase inhibition was evaluated by Young *et al*., 2008, method with revision [13-15]. Indus Viva I Pulse health drink extract was taken in each test tube at various concentrations of 500, 250, 100, 50 and 10 µg/mL of eucalyptus. The medium was prepared (starch, phosphate and DNS) and incubated at 25°C for 30 min (pH 6.9). Control incubations representing 100% enzyme activity were conducted similarly by replacing extracts with buffers [16]. The α-α-amylase inhibitory activity was calculated as follows:

$$\%\ Inhibition = \frac{A\ control - (A\ test - A\ background)}{Control} \times 100$$

Methyl-thiazolyl-tetrazolium (MTT) assay for cell cytotoxicity

The anticancer activity of Indus Viva I Pulse health drink extracts from herbal medicine was studied against bone cancer (MG-63) cell lines sourced from the National Centre for Cell Sciences, Pune, India. The cell lines were cultured in fetal bovine serum (FBS) as an essential media, further supplemented with 10% heat-inactivated (FBS), penicillin (100 µg/mL), and streptomycin (100 µg/mL) in a humidified atmosphere of 50 µg/mL CO_2 at 37°C. Anticancer activity of Indus Viva

Table 1: Total phenol and flavonoid content of Indus Viva I Pulse syrup

Total phenol content				Total flavonoid content			
Name of the sample	**OD value at 750 nm**	**Total Phenol content**	**Mean value of total phenol content**	**Name of the sample**	**OD value at 750 nm**	**Total flavonoid content**	**Mean value of total flavonoid content**
Indus Viva I Pulse	0.213 0.203 0.300	0.11 0.09 0.29	0.16	Indus Viva I Pulse	0.669 0.390 0.421	0.76 0.13 0.20	0.36

Table 2: HPLC profile of Indus Viva I Pulse syrup

Herbs	**Indus Viva I Pulse**			
S. No.	**RT**	**Area**	**% Area**	**Height**
1.	2.436	4063732	9.03	210.2
2.	2.830	6018990	13.37	210.2
3.	4.818	2223173	4.94	210.2
4.	5.034	4212258	9.36	210.2
5.	8.533	24162131	53.67	258.3
6.	9.953	4343025	9.65	210.2

HPLC: High-performance liquid chromatography

I Pulse health drink extracts at various concentrations (500, 400, 300, 200, 100, 80, 60, 40, 20 and 10 µg/mL) was assessed using trypsin, methyl thiazole, diphenyl-tetrazolium bromide (MTT) (Sigma) assay, as described by Mosmann, but with some slight modifications involving an incubation period of 24–48 h at 37°C. Then, the cells were plated at a density of 1 × 10^5 cells/mL into the 96-well tissue culture plate in a DMEM medium. The absorbance for each well was measured at 570 nm using a microplate reader and the percentage cell viability and IC_{50} value were calculated using GraphPad Prism 6.0 software (USA) [17].

Formula cell viability % = *Test OD*/*Control OD* × 100

RESULTS AND DISCUSSION

Quantitative identification test result for phenol and flavonoids

The total phenol and flavonoid content present in the extracted sample Indus Viva I Pulse health drink were detected to be 0.16 and 0.36 mg/mL, respectively. The total phenol and flavonoid content calculation of Indus Viva I Pulse health drink is given in Table 1.

HPLC analysis

Herbal medicine Indus Viva I Pulse health drink (methanol/H_2O, 50:50, v/v) was investigated by HPLC which showed RT values between about 2.436 and 9.953 min for Indus Viva I Pulse health drink. RT values and area indicated that phytochemical constituents, namely flavonoid, alkaloid, ester, phenol, acid, carboxylic acid, fatty acid and carbohydrate were present in Indus Viva I Pulse health drink. Compound RT values are identical to those of the standards. HPLC Chromatograms of Indus Viva I Pulse health drink are shown in Fig. 1 and their values are given in Table 2.

GC-MS analysis

Herbal medicine Indus Viva I Pulse health drink gas chromatography mass-spectrometry chromatogram (1I–12I) is shown in Fig. 2. Retention time value, % peak area, peak area, molecular formula, compound name, molecular weight (m/z), compound nature and biological activity are shown in Table 3. GC-MS results (Fig. 2) showed 12 peaks that indicated 12 phytoconstituents, namely 2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one, benzyl alcohol, 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, glycerin, 5-hydroxymethylfurfural, melezitose, 7-methyl-Z-tetradecen-1-ol acetate, 3-butyl-4-nitro-pent-4-enoic acid, methyl ester, n-hexadecanoic acid, trans-13-octadecenoic acid, hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, octadecanoic acid and 2-hydroxy-1-(hydroxymethyl)ethyl ester, were found in Indus Viva I Pulse health drink (1I–12I). Herbal medicine Indus Viva I Pulse health drink is used to cure a variety of diseases like diabetics, loss of sight, cancer, tuberculosis, antimalarial, antiviral, antibacterial, analgesic,

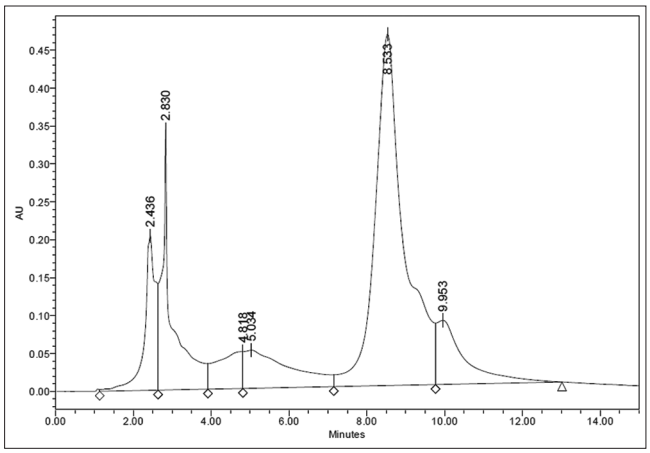


Fig. 1: High-performance liquid chromatography of Indus Viva I Pulse herbal medicine

antimicrobial and antifertility which is shown in Table 3. Phytochemical and biological studies of this herbal medicine have not been reported. Herbal medicines are sources for Ayurveda, Naturopathy, Unani, Siddha, and Homeopathy treatments which possess fewer side effects and huge healing power, Continued further exploration of herbal medicine is needed today. Results of GC-MS of herbal Indus Viva I Pulse health drink (1I–12I) are given in Table 3 and Fig. 2.

Spectral studies

Spectroscopic studies and Liquid Chromatography - High-Resolution Mass Spectrometry [LC-HRMS]) of the herbal medicine Indus Viva I Pulse health drink were carried out. IR (KBr, cm^{-1}): 3457, 2922, 2857, 2121, 1641, 1055, 1033 and 621 cm^{-1}. UV/Vis (CH_3OH): λmax (ε) = 319 and 412 nm. ^{1}H NMR (400 MHz, DMSO):δ = 5.30 (s, Aromatic), 4.05 (s, 1H), 3.60 (d, 1H), 3.40 (m, 1H), 3.00 (s, 1H), 2.50 (s, 1H). m/z: 1134. 7017, 1150.6475 and 1168.6797. Herbal medicine Indus Viva I Pulse Health Drink has the following spectral characteristics. It is a polyherbal formulation of brown-colored liquid. Its UV spectrum in methanol is characteristic of an aromatic compound with λmax of 319 and 412 nm. IR spectrum gives peaks at 3457, 2922, 2857, 2121, 1641, 1055, 1033 and 621 cm^{-1} indicating the presence of an alcoholic, alkyl, aldehyde or ketone, alkyne, carbonyl, anhydride and methyl group respectively. It gave molecular ions at m/z values: 1134, 7017, 1150.6475 and 1168.6797 with aromatic characteristics also UV spectrum and IR spectra indicating an aromatic nature [18]. ^{1}H NMR indicates the presence of aromatic protons and methyl protons. IR and UV spectra are shown in Fig. 3a and b. ^{1}H NMR spectrum is shown in Fig. 4.

LC-HRMS analysis of Indus Viva I Pulse health drink

LC-HRMS is the best tool for measuring the molecular weight of new drug entities as part of the novel medicine research design and evolution system that is also used for bioanalysis in the pharmaceutical industry. HR-LCMS gave a qualitative and quantitative analysis of molecular weight with corresponding molecular ion peaks of medicinal substances such as anabolites, degenerates, immunomodulation, and immunotherapy. In this aspect, LC-HRMS of Indus Viva I Pulse health drink was carried out by optimizing through the solvent, flow rate, scan speeds, pressure,

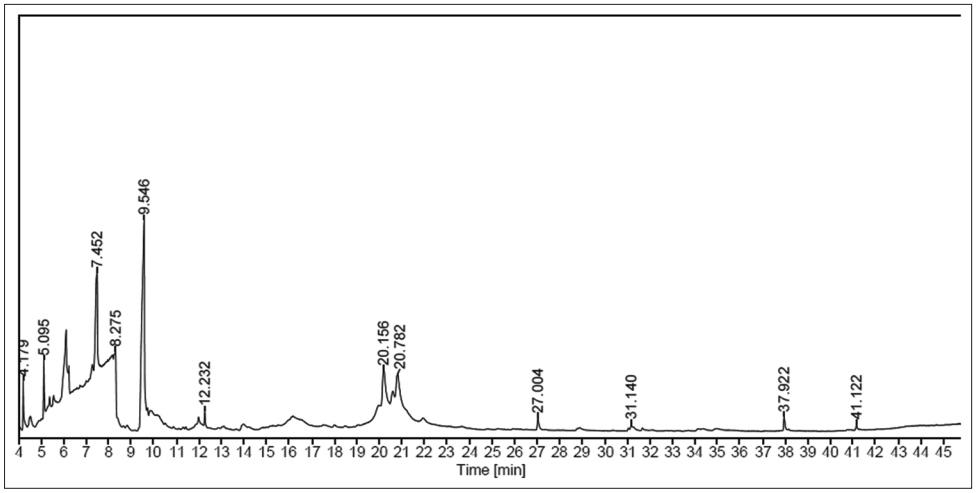


Fig. 2: Gas chromatography–mass spectrometry chromatogram of Indus Viva I Pulse herbal medicine

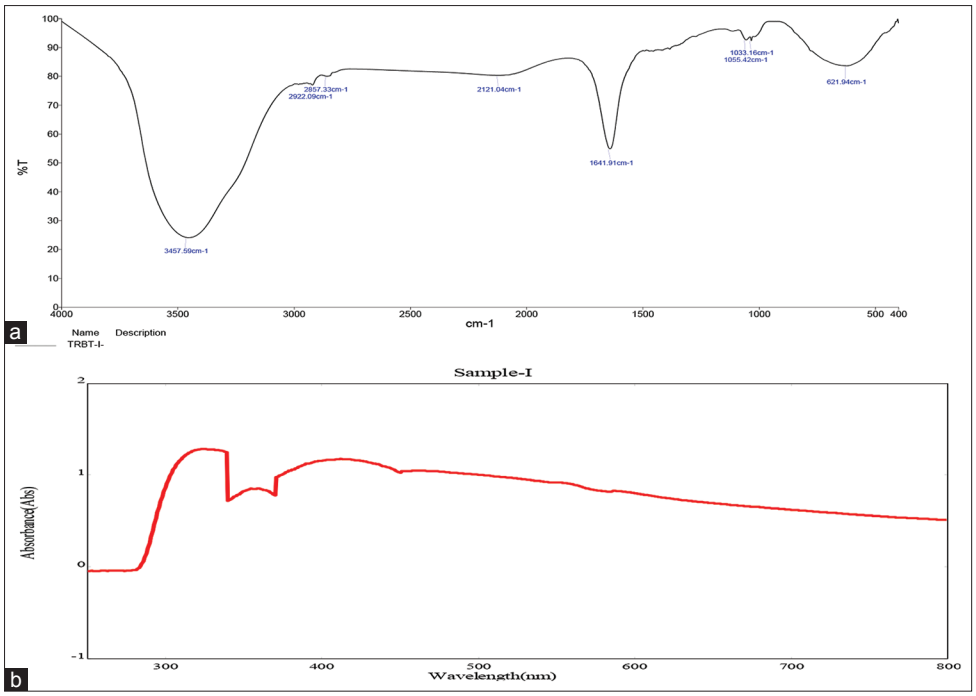


Fig. 3: (a) Infrared spectrum of Indus Viva I Pulse. (b) Ultraviolet spectrum of Indus Viva I Pulse

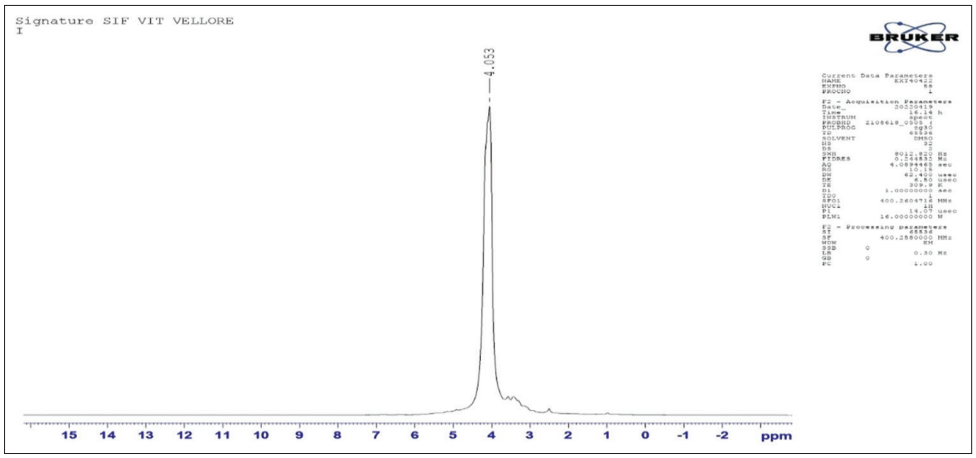


Fig. 4: ^{1}HNMR spectrum of Indus Viva I Pulse

Table 3: GC-MS investigation of herbal medicine Indus Viva I Pulse syrup (1I–12I)

S. No.	Retention Time (min)	Peak area	Peak area %	Peak @	Molecular Weight m/z	Molecular formula	Compound name	Compound nature	Biological activity
1I	4.181	2999743.784	3.65	4.179	144.0	$C_6H_8O_4$	2,4-dihydroxy-2,5-dimethyl-3 (2H)-furan-3-one	Flavonoid/ Furanones/ furaneol	Anti-inflammatory AND Antioxidant agents
2I	5.094	3125084.314	3.80	5.095	108.0	C_7H_8O	Benzyl alcohol	Alcohol	Antimicrobial and antibacterial agents
3I	7.450	14153376.783	17.21	7.452	144.0	$C_6H_8O_4$	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	Flavonoid	Anti-microbial, anti-inflammatory, and strong antioxidant capacity properties
4I	8.275	4195671.861	5.10	8.275	92.09	$C_3H_8O_3$	Glycerin	Sugar alcohol	Antimicrobial and antibacterial agents
5I	9.544	37426945.233	45.52	9.546	126.0	$C_6H_6O_3$	5-hydroxymethylfurfural	Reducing sugars	Pharmacological activities such as sickle-cell anemia and type I allergic reactions, antimicrobial activity, and protecting liver and kidney. Antioxidant and anticancer activity.
6I	12.232	3204294.803	3.90	12.232	504.44	$C_{18}H_{32}O_{16}$	Melezitose	Non-reducing trisaccharide sugar	Glucosyltransferase activity
7I	20.159	3068437.415	3.73	20.156	268.0	$C_{17}H_{32}O_2$	7-Methyl-Z-tetradecen-1-ol acetate	Acetate ester	Antimicrobial and antioxidant agents
8I	20.784	8811871.759	10.72	20.782	215.0	$C_{10}H_{17}NO_4$	3-Butyl-4-nitro-pent-4-enoic acid, methyl ester	Methyl Ester	Antimicrobial and antibacterial agents
9I	27.004	1485058.085	1.81	27.004	256.0	$C_{16}H_{32}O_2$	n-Hexadecanoic acid	Saturated fatty acids	Antioxidants, hypocholesterolemic, nematicide, and pesticide
10I	31.140	1687400.554	2.05	31.142	282.0	$C_{18}H_{34}O_2$	Trans-13-Octadecenoic acid	Saturated fatty acids	Antimicrobial activity
11I	37.922	1647868.840	2.0	37.925	330.0	$C_{19}H_{38}O_4$	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	Fatty acid ester	Hemolytic, pesticide, flavor, and antioxidant agents
12I	41.125	413767.848	0.50	41.122	358.0	$C_{21}H_{42}O_4$	Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	Acid ester	Antioxidant, anti-inflammatory, and antibacterial activity

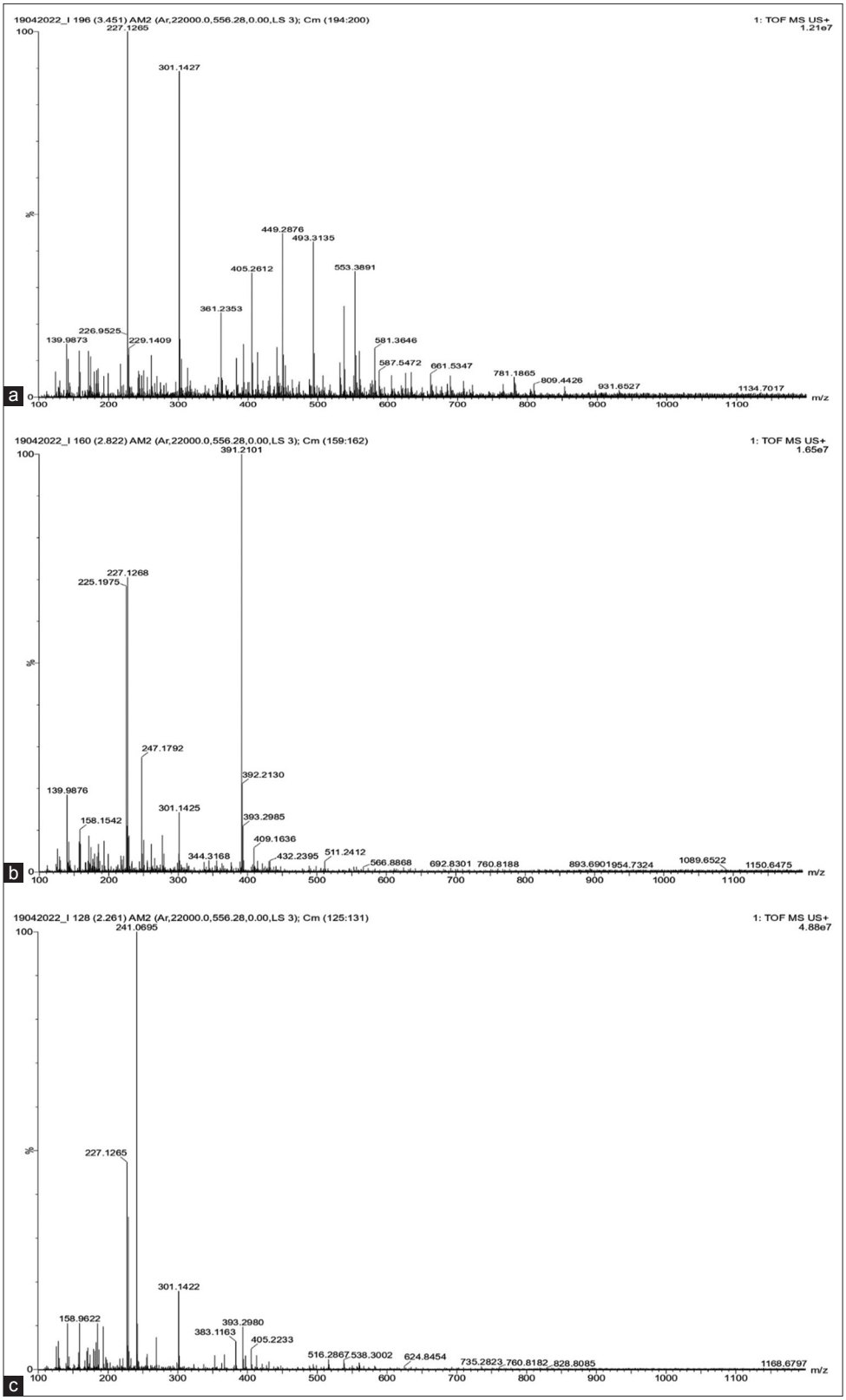


Fig. 5: (a) LC-HRMS Mass Spectrum of Indus Viva I Pulse. (b) LC-HRMS Mass Spectrum of Indus Viva I Pulse. (c) LC-HRMS Mass Spectrum of Indus Viva I Pulse

and time. The analytical LC-HRMS experiments were performed using a mixture of acetonitrile, water and formic acid at a flow rate of 0.4 mL/min. Before the experiment, m/z value from 100 to 1200 various desolvation temperature at 1.21e+7, 4.88 e+7 and 1.65 e+7 was selected for the acquisition of accurate mass precursor and fragment ion data.

Indus Viva I Pulse health drink was investigated through LC-HRMS with Ar 2200 by orthogonal time-of-flight SIMS using bombarding energies 1.21e+7, 4.88 e+7 and 1.65 e+7. The first scan at low collision energy (1.21 e+7) results in an MS full scan over the range m/z 100–1200. The second scan at high collision energy (4.88 e+7)

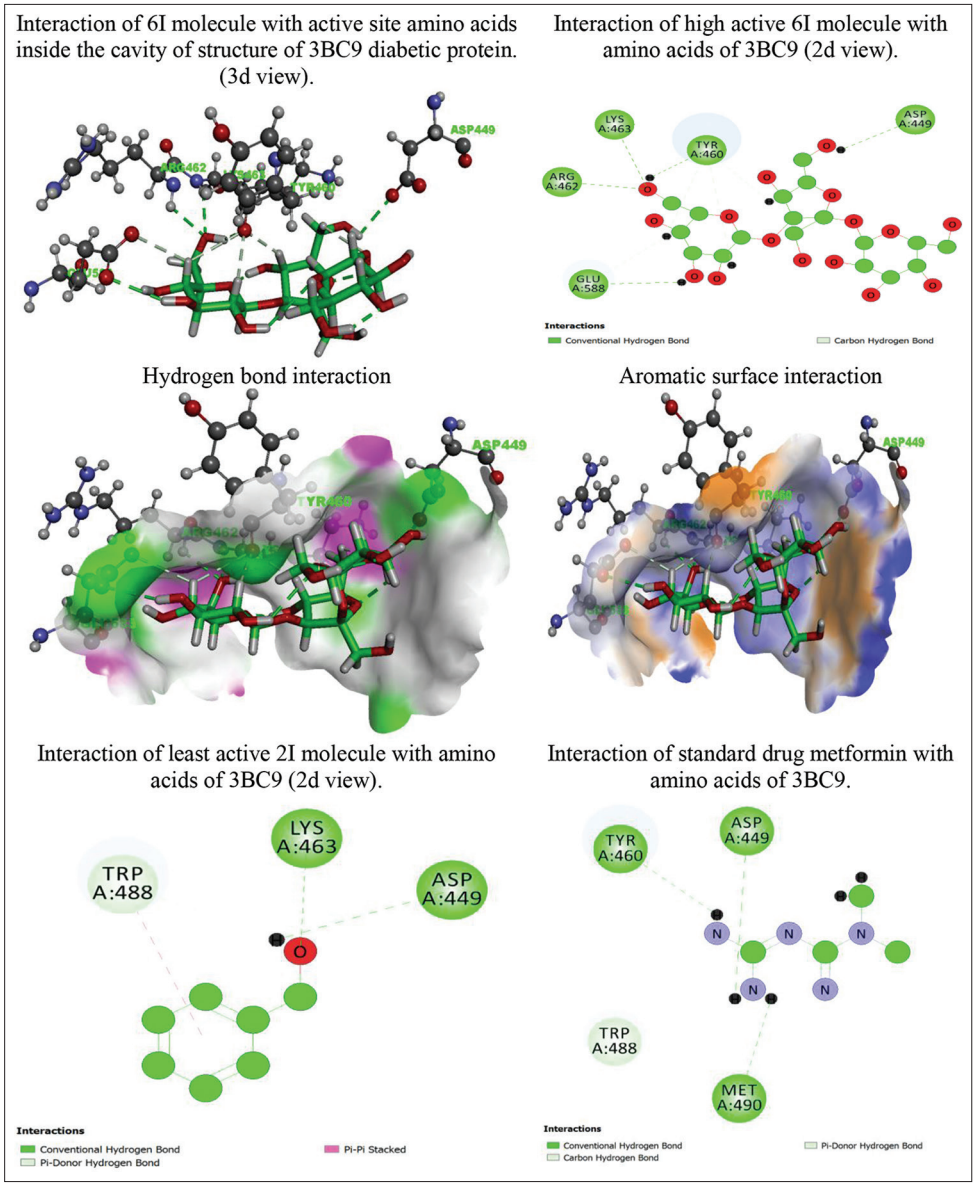


Fig. 6: Docking studies reported image of natural ayurvedic Indus Viva I Pulse syrup with 3bc9 diabetic protein

Table 4: SD±Means of a zone of inhibition obtained by sample herb Indus Viva I Pulse against given types of organisms

S. No	Name of the test organism	Name of the test sample	Zone of inhibition (mm) SD±Mean				
			PC	500 µg/µL	250 µg/µL	100 µg/µL	50 µg/µL
1.	*Escherichia coli*	Indus Viva I Pulse	18.5±0.7	13±0	11.5±0.7	7.5±0.7	0
2.	*Staphylococcus aureus*		20±0	12.5±0.7	11.5±0.7	8.5±0.7	0
3.	*Aspergillus niger*		16.5±0.7	13±1.4	11.5±0.7	10.5±0.7	0
4.	*Candida albicans*		17.5±0.7	13.25±0.35	12.5±0.7	11±0	8.5±0.7

*Significance - $p<0.05$. SD: Standard deviation

Table 5: Percentage of inhibition of α-amylase inhibitory activity

S. No.	Tested sample concentration (µg/mL)	Percentage of inhibition (in triplicates)			Mean value (%)
1.	500	74.53	74.42	74.27	74.41±0.075
2.	250	74.01	73.64	73.49	73.71±0.155
3.	100	69.9	69.54	69.85	69.76±0.113
4.	50	68.08	68.97	68.55	68.533±0.257
5.	10	54.1	51.6	53.5	53.067±0.754
6.	Acarbose µg/mL	80.19	78.06	78.5	78.917±0.649

*t-test P value (Two tailed) < 0.0001. Significant (α=0.05) yes, r^2=0.9872.

Table 6: Results of cell viability (MTT assay for cell cytotoxicity of human bone cancer cells)

S. No.	Tested sample concentration (µg/mL)	Cell viability (%) (in triplicates)			Mean value (%)
1.	Control	100	100	100	100
2.	500	1.12179	4.73282	3.45912	3.1045796±1.057
3.	400	5.44872	7.32824	11.3208	8.0325723±1.731
4.	300	21.6346	17.0992	13.0503	17.261389±2.479
5.	200	28.2051	20.3053	19.9686	22.826342±2.691
6.	100	39.9038	31.7557	33.805	35.154868±2.447
7.	80 µ	39.5833	40	44.9686	41.517296±1.730
8.	60	56.25	54.8092	53.7736	54.944248±0.718
9.	40	60.7372	55.2672	61.7925	59.265603±2.022
10.	20	77.8846	69.0076	74.8428	73.911672±2.605
11.	10	94.391	86.7176	91.5094	90.872672±2.238

t-test P value (two-tailed)<0.005. Significant (α=0.05) yes, r^2=0.8868,

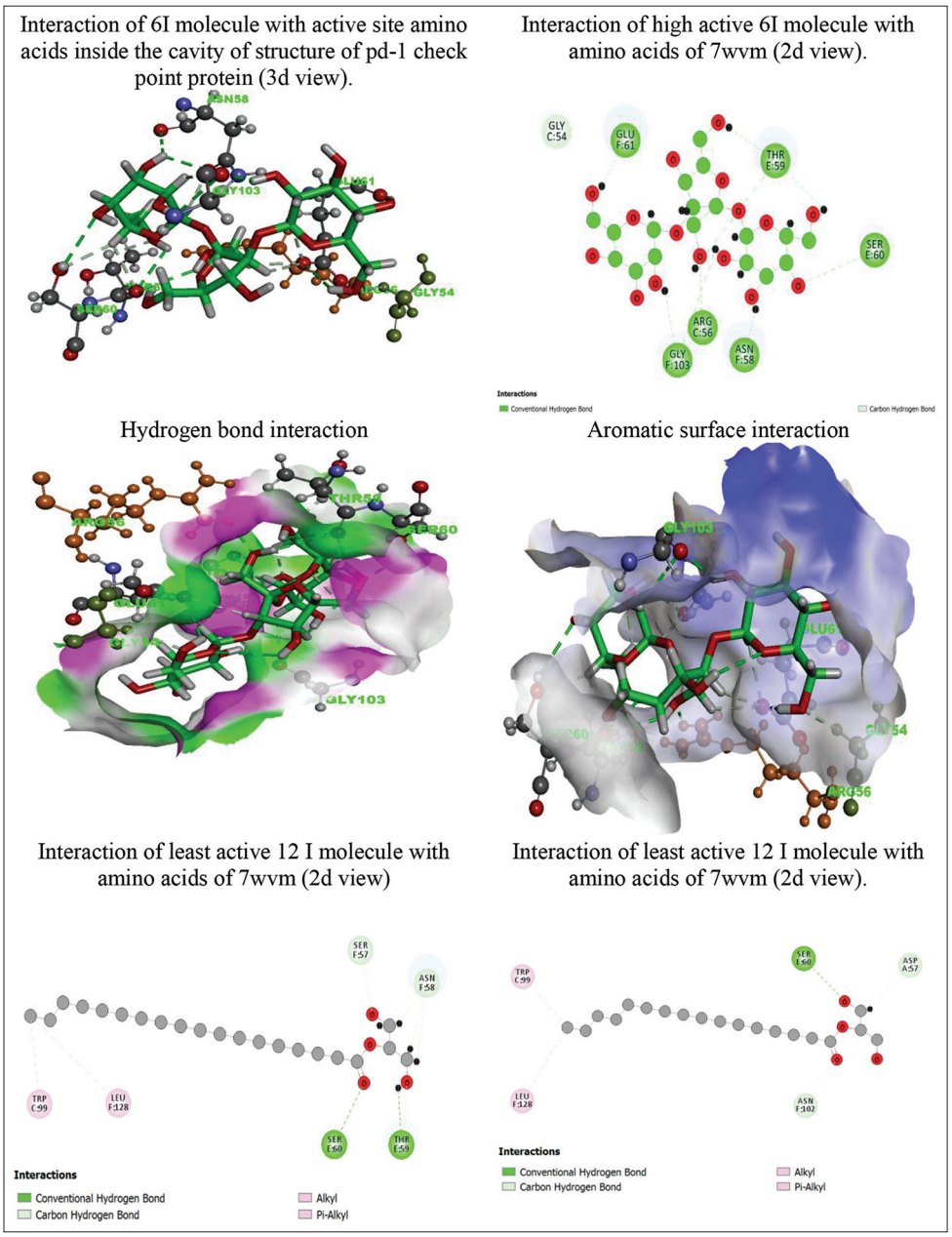


Fig. 7: Docking studies reported image of natural ayurvedic Indus Viva I Pulse syrup with 7 wvm bone cancer

results in an MS/MS all ion fragment mode also in the range m/z 100–1200. The scan rate was 2 Hz/cycle. The third scan at low collision energy (1.65 e+7) results in an MS full scan over the range m/z 100–1200. It gave molecular ions at m/z 1134. 7017, 1150.6475 and 1168.6797. LC-HRMS mass spectra of the compound are given in Fig. 5a-c.

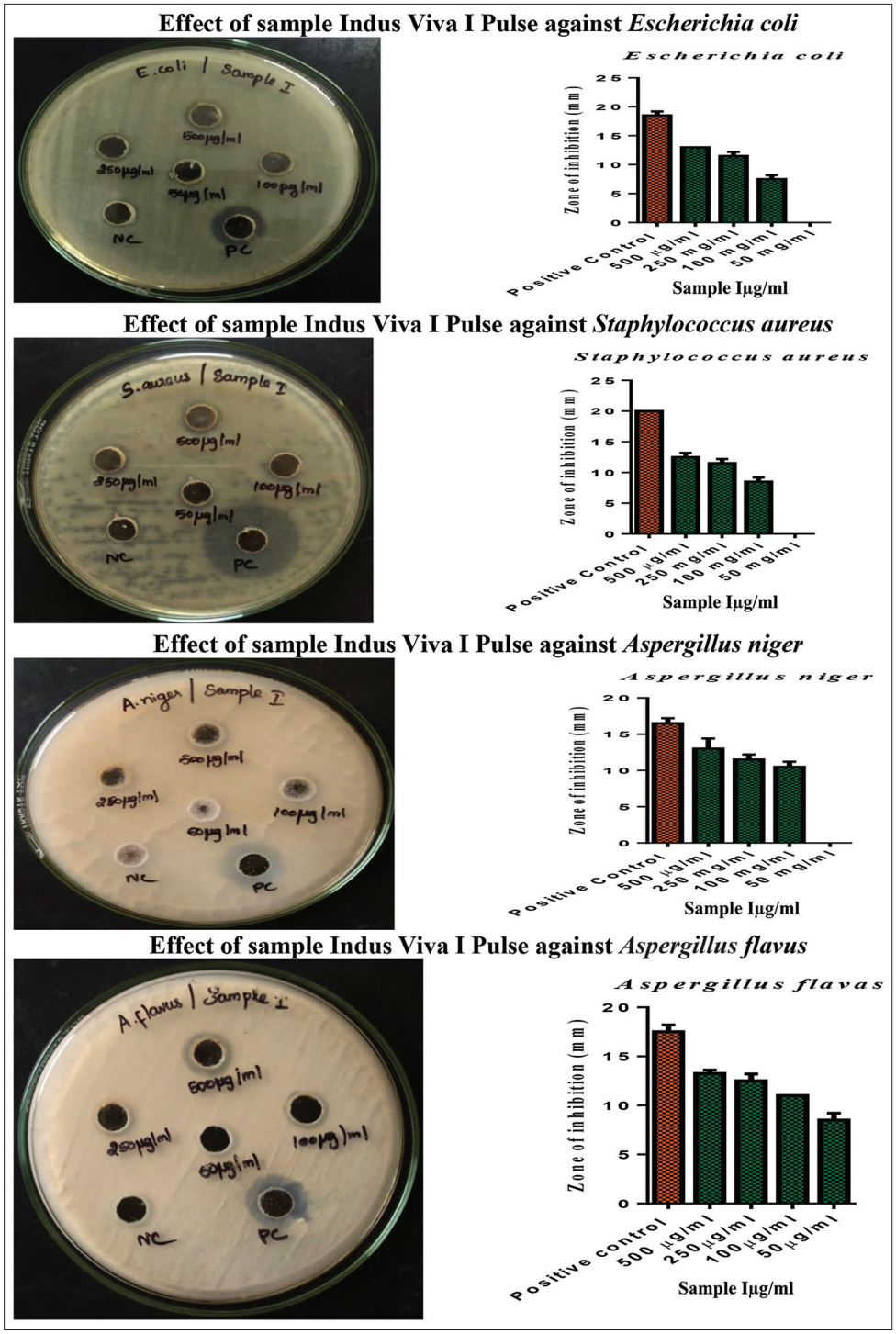
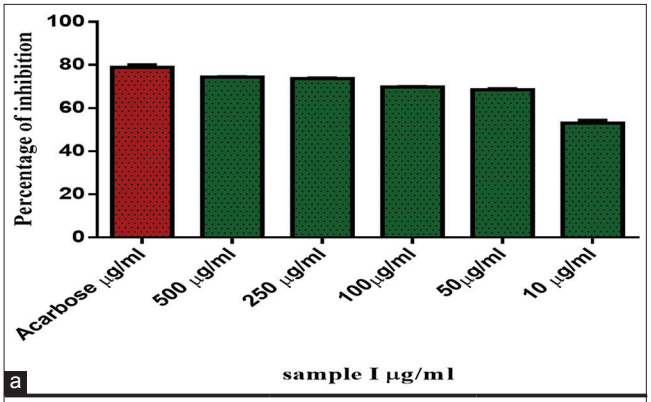


Fig. 8: Antimicrobial images of herb medicines Indus Viva I Pulse

Molecular docking studies

Indus Viva I Pulse health drink 12 phyto-compounds docked against 3BC9 diabetic protein and bone cancer protein 7WVM were discovered to show a very admirable docking score. Binding interactions with diabetic protein 3BC9 of the following compounds 1I, 2I, 3I, 4I, 5I, 6I, 7I, 8I, 9I, 10I, 11I and 12I showed 4.20233, 15.8854, 11.7932, 11.407, 21.8406, –21.4371, 28.2226, 17.7428, 40.2692, 31.1641, 43.5513 and 46.9598 scores respectively. All phytoconstituents showed RMSD values around 0.08484. There are great binding scores when compared with the standard drug for diabetics – metformin – whose docking score is 12.8149. The docking result of melezitose (6I) exhibited the highest docking score (–21.4371 kcal/mol) against 3BC9 diabetic protein in the present investigation. It was found in the study that, the melezitose compound is bound with glutamine, arginine, lysine, tyrosine and aspartic amino acids. Furthermore, the bone cancer protein 7WVM binding score energy results are 35.1348, 50.7744 and 55.1057 corresponding to phytocompounds from 1I to 12I. Here all phytoconstituents showed RMSD values are around 0.00963. Melezitose compound showed a docking score of –9.59309 which makes it the best agent for inhibition of bone cancer protein 7WVM. In docking studies between Indus Viva I Pulse health drink 12 phytoconstituents and bone cancer protein 7WVM, melezitose compound bound with the following amino acids as asparagine, arginine, glycine, glutamate, threonine, and serine. Overall docking results show that melezitose possessed a



a

Log (inhibitor) vs. normalized response -- Variable slope	
Best-fit values	
$LogIC_{50}$	1.558
HillSlope	−1.998
IC50	36.18 µg/mL
Std. Error	
$LogIC_{50}$	0.04265
HillSlope	0.3186
95% Confidence Intervals	
$LogIC_{50}$	1.466–1.651
HillSlope	−2.686–−1.310
IC_{50}	29.26–44.72
Goodness of Fit	
Degrees of Freedom	13
R square	0.9650
Absolute Sum of Squares	694.2
Sy.x	7.308
Number of points	

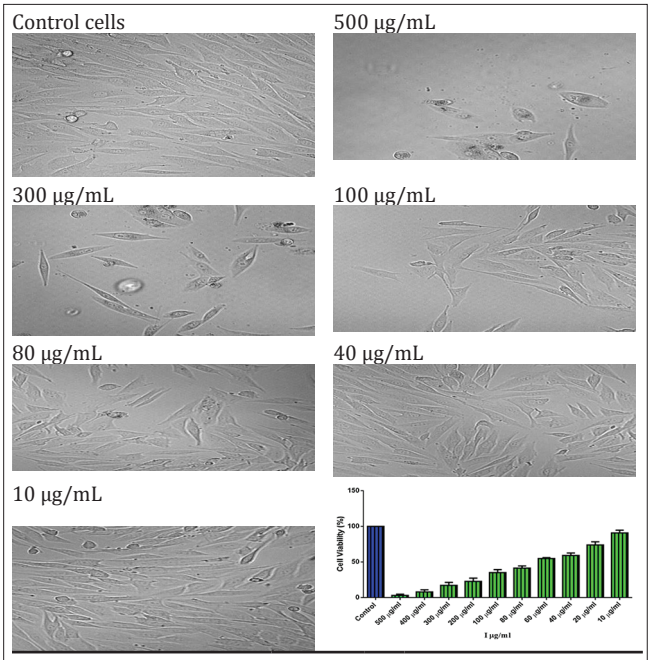
b

Fig. 9: (a) α-amylase inhibitory activity was expressed in Graph. (b) IC_{50} Value of tested sample: 36.18 µg/mL

strong correlation binding affinity with 3BC9 diabetic protein and bone cancer protein 7WVM among the 12 phytoconstituents. Thereby, these phytochemicals composed in Indus Viva I Pulse, jointly have the potential to inhibit the 3BC9 diabetic protein and bone cancer protein 7WVM. The best docking score models are shown in Figs. 6 and 7. 2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one (1I), benzyl alcohol (2I), 3,5-dihydroxy-6-methyl 2H-pyran-4(3H)-one, 2,3-dihydro- (3I), glycerine (4I), 5-hydroxymethylfurfural (5I), melezitose (6I), 7-methyl-Z-tetradecen-1-ol acetate (7I), 3-butyl-4-nitro-pent-4-enoic acid, methyl ester (8I), n-hexadecanoic acid (9I), trans-13-octadecenoic acid (10I), hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (11I) and octadecanoic acid 2-hydroxy-1-(hydroxymethyl)ethyl ester (12I) are compounds which have 4,1,4,3,3,16,1,4,2,2,4 and 4 hydrogen bond acceptor atoms and 2,1,2,3,1,11,0,0,1,1,2 and 2 hydrogen bond donor atoms and 0,1,0,2,2,8,13,8,14,15,18, and 20 rotatable bonds respectively [19].

Antibacterial and antifungal activity of Indus Viva I Pulse health drink

In vitro antimicrobial studies were carried out by the disk-diffusion method as described previously with some modifications (Murray *et al*., 1995) [20]. Gentamicin antibiotic was used as a positive control for bacteria. Amphotericin was used as a positive control for fungi. The following bacteria and fungi were used for the experiment, Bacteria such as *E. coli* and *S. aureus* and fungi, namely *A. niger* and *C. albicans.* To determine the antibacterial activity of water extract of herbal medicine Indus Viva I Pulse health drink, we evaluated the effect of herbal medicine Indus Viva I Pulse health drink extract on the growth of *S. aureus* is a gram-positive (*S. aureus*) and gram-negative bacteria include *E. coli*. Herbal medicine Indus Viva I Pulse health drink exhibited antibacterial activity; however, not all of them reached positive control values (Vineet, *et al*., 2013) [21]. Herbal medicine Indus Viva I Pulse



log(inhibitor) versus normalized response -- variable slope		
Best-fit values		
$LogIC_{50}$		1.839
HillSlope		−1.360
IC50		68.95 µg/mL
Std. Error		
$LogIC_{50}$		0.01768
HillSlope		0.07743
95% Confidence Intervals		
$LogIC_{50}$		1.802–1.875
HillSlope		−1.518–−1.201
IC50		63.43–74.94
Goodness of Fit		
Degrees of Freedom		28
R square		0.9751
Absolute Sum of Squares		738.8
Sy.x		5.137
Number of points		
Analyzed	3	30

IC_{50} Value of tested sample: 68.95 µg/mL

Fig. 10: Images and graphs of cell viability of human bone cancer cells

health drink extract showed the highest growth inhibitory activity against gram-positive (PC >500 µg/µL >250 µg/µL >100 µg/µL >50 µg/µL) and -negative bacteria ((PC >500 µg/µL >250 µg/µL >100 µg/µL >50 µg/µL). The highest inhibition of *E. coli* growth was observed at 500 µg/µL Indus Viva I Pulse health drink extract. Moderate inhibition of *S. aureus* growth was observed at 500 µg/µL Indus Viva I Pulse health drink extract. In contrast, *E. coli* growth was little affected by Indus Viva I Pulse health drink extract (100 µg/µL). Indus Viva I Pulse health drink water extract was the greatest potent inhibitor of fungal growth. To learn the antifungal activity of the herbal medicine Indus Viva I Pulse health drink, we evaluated the effect of herbal extracts on the growth of the fungi, *A. niger* and *C. albicans*. All extracts showed fungal growth inhibitory activity [22]. Indus Viva I Pulse health drink water extract was more susceptible to *A. niger* in an aqueous extract at 500, 250, 100, and 50 µg/µL. Indus Viva I Pulse health drink herb extract exhibited the strongest antifungal activity against *A. niger* and *C. albicans*. The herb extract showed different degrees of inhibitory activity on *A. niger* and *C. albicans* growth: *A. niger*: 13±1.4 mm (500 µg/µL), 11.5±0.7 mm (250 µg/µL), 10.5±0.7 mm (100 µg/µL), and 0 mm (50 µg/µL) and *C. albicans:* 13.25±0.35 mm (500 µg/µL), 12.5±0.7 mm (250 µg/µL), 11±0 mm (100 µg/µL) and 8.5±0.7 mm (50 µg/µL). The herbal medicine

Indus Viva I Pulse health drink syrup did not show any activity against *A. niger* (fungal) in 50 µg/µL. But herbal medicine Indus Viva I Pulse health drink showed an inhibition value of 8.5±0.7 mm that revealed activity against *S. aureus* (gram-positive bacterium) at 50 µg/µL. Also, the herbal medicine Indus Viva I Pulse health drink showed an inhibition value of 8.5±0.7 mm that revealed activity against *C. albicans* (fungal) at 50 µg/µL. Herbal medicine Indus Viva I Pulse health drink extract exhibited admirable inhibitory activity on *A. niger* and *C. albicans*. The antibacterial and antifungal activity of Indus Viva I Pulse health drink is given in Table 4 and Fig. 8.

Appraise herb Indus Viva I Pulse health drink extract used in ∞ - Amylase inhibitory assay (Diabetic) clinical experiment

In this experiment, the herbal medicine Indus Viva I Pulse health drink extract was investigated along with the commercially available antidiabetic drug acarbose to find an α-amylase inhibition assay which is a simple technique to confirm the antidiabetic activity of Indus Viva I Pulse health drink. The main examination of α-amylase inhibition is ground on the compound formation between starch-iodine and Indus Viva I Pulse health drink extract. The anti-diabetic activity of Indus Viva I Pulse health drink extract was analyzed by performing an α-amylase inhibition assay described by the standard procedure which was given in the materials and method. Indus Viva I Pulse health drink extract α-amylase inhibition is minimal at low concentrations and maximal at high concentrations respectively [23]. The percentage inhibitory activity exhibited by Indus Viva I Pulse health drink extract and standard drug acarbose is shown in Table 5 and Fig. 9a and b. α-Amylase inhibition results are 74.41%, 73.71%, 69.76%, 68.53% and 53.10% corresponding concentrations with 500, 250, 100, 50, and 10 µg/mL. Furthermore, Acarbose showed an α-amylase inhibition result is 78.93% and Indus Viva I Pulse health drink syrup extract showed the highest inhibition value is 74.41 % at 500 µg/mL with an IC_{50} value is 36.18 µg/mL. Herbal medicine Indus Viva I Pulse health drink extract possesses anti-diabetic potential based on concentration [24].

Evaluate herb Indus Viva I Pulse health drink extract used in Osteosarcoma clinical investigation

The cytotoxicity of the herb Indus Viva I Pulse health drink extract against human bone cancer cell line (MG-63) was assessed using the MTT Mosmann and Marshall model. Anticancer studies *in vitro* in human bone cancer cells (MG-63) are carried out and are shown in Fig. 10. Results showed that inhibition of proliferation is between 3.11% and 90.87% corresponding concentrations from 500 µg/mL to 10 µg/mL in *in vitro* in human bone cancer cells (MG-63) (Prasanth *et al*., 2010). MTT assay results showed the lowest proliferation value is 90.87% at 10 µg/mL with an IC_{50} value is 68.95 µg/mL. Therefore, the study suggested that Indus Viva I Pulse health drink is a moderate potential therapeutic agent for human bone cancer cells (MG-63). The anticancer activity of Indus Viva I Pulse health drink syrup is given in Table 6 and Figure 10. Results revealed inhibition of proliferation to be around 3.10 % to 90.87% in the bone cancer cells (MG-63) line at the administered dose of 3.10% (500 µg/mL), 8.03% (400 µg/mL), 17.26% (300 µg/mL), 22.83% (200 µg/mL), 35.15% (100 µg/mL), 41.52% (80 µg/mL), 54.94% (60 µg/mL), 59.27% (40 µg/mL), 73.91% (20 µg/mL) and 90.87% (10 µg/mL) thereby proving to be moderate anticancer potential Indus Viva I Pulse health drink extract whose bone cancer IC_{50} value ranging between 63.43 to 74.94 µg/mL was observed upon administration of this Indus Viva I Pulse health drink. From the literature, we observed that standard drugs adiromycin, doxorubicin, and cisplatin whose IC_{50} values vary with different concentration, experimental duration as well as experimental technique. This study shows that dose responses characterized proliferation activity by quantitatively resistant properties. MTT assay results exhibit the highest proliferation value is 3.10% at 500 µg/mL, moderate proliferation value is 35.15 % at 100 µg/mL, and lowest proliferation value is 90.87 % at 10 µg/mL. IC_{50} value is 68.95 µg/mL at (halfway point or 50%) average µg/mL. This study exposed that at high concentrations Indus Viva I Pulse health drink produces a greater amount of cellular debris. Results of the current study showed that Clutch was inspired by a new anticancer herb medicine Indus Viva I Pulse health drink [24-26]. Furthermore, fast and convenient studies are needed to determine the osteosarcoma clinical investigation of Indus Viva I Pulse health drink.

CONCLUSION

Indus Viva I Pulse health drink syrup extract was screened by *in vitro* antibacterial, antifungal, anti-diabetic and anticancer (bone cancer) activities as well as characterized by Fourier transform-IR, UV, ^{1}HNMR, ^{13}C NMR and Mass spectrometry (LC-HRMS) studies and bio-active compounds were detected after HPLC and GC-MS. Further, this herbal medicine underwent antimicrobial activity through fungi and bacteria. This herbal medicine reports a higher zone of inhibition when compared to the standard drug. The results of the present study indicate that the aqueous extract of Indus Viva I Pulse health drink syrup showed the maximum alpha-amylase inhibitory activity. Indus Viva I Pulse health drink syrup contains herbal bioactive compounds inhibiting enzyme activity. In conclusion, Indus Viva I Pulse health drink syrup extract has more inhibitory properties against alpha-amylase than a standard drug named Metformin. This medicine is used as a drug against diabetes. From these results, we find that Indus Viva I Pulse health drink syrup serves well in chemopreventive treatment with therapeutic potential against bone cancer. Moreover, the separation of individual biochemical compounds from Indus Viva I Pulse health drink syrup extract undergoes pharmacological studies to be provide an efficient outcome and open research area of expertise.

ACKNOWLEDGMENT

The authors are thankful to the Chairman, Honorable Secretary and Correspondent, Principal, Vice-Principals (Academic and Administration) and Head, P.G. and Research Department of Chemistry, The New College (Autonomous), Chennai for their constant support and encouragement.

AUTHOR'S CONTRIBUTIONS

The concept and design of the study, data collection, data analysis, and manuscript writing were done by the first and corresponding author. Data curation, data validation, software, statistical analysis, review, and editing were done by second and third author. All authors have read and agreed to the published version of the manuscript.

CONFLICTS OF INTEREST

We declare that we have no conflict of interest.

FUNDING AND SUPPORT

It is declared that the authors did not receive any financial support or sponsorship for their study.

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