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RESEARCH ARTICLE

ISOLATION OF VITEXIN FROM METHANOLIC EXTRACT OF BEET ROOT (BETA VULGARIS) AND EVALUATION OF IN VIVO ANTITUMOUR ACTIVITY AGAINST S-180 AC RAT MODEL FOLLOWED BY MOLECULAR DOCKING AGAINST TOPOISOMERASE-I

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ABSTRACT

The main objective of the present research work was the isolation of the bioactive molecule vitexin present in the methanolic extract of beet root *(Beta vulgaris)* and evaluation of antitumor activity. Vitexin was characterized by HPLC-MS, IR, H^1-NMR, ^{13}C-NMR etc. Molecular docking of vitexin with respect to the target protein Topoisomerase-I was evaluated by Auto dock program with PDB id 1A36 and displayed the binding energy -3.95 k.cal/mol. In vivo antitumor activity of ME-BRT was carried out against S-180-AC rat model. ME-BRT (100 mg/kg), ME-BRT (200 mg/kg) significantly increased the PILS. While topotican increased the life span of S-180-AC 78.57%, ME-BRT (100 mg/kg) increased it by 57.14% and 71.42 % ME-BRT (200 mg/kg) respectively. So ME-BRT at dose 100 and 200 mg/kg significantly improved the overall survival of all treated animals and topotecan was not significantly differed from each other in improving the overall survival of S-180-AC. Both extracts displayed a significant inhibition of tumour growth at doses of 100 and 200 mg/kg with values of 81.90 and 88.33%, respectively, with no mortality, compared standard drug topotecan (95.94%).

INTRODUCTION

The roots and leaves of the beet have been used in folk medicine to treat a wide variety of ailments (Edgar J. DaSilva *et al*., 2002). Ancient Romans used beetroot as a treatment for fevers and constipation, amongst other ailments. Apicius in De re coquinaria gives five recipes for soups to be given as a laxative, three of which feature the root of beet (http://www.nutrition-and-you.com/beets.html). Hippocrates advocated the use of beet leaves for binding wounds. Since Roman times, beetroot juice has been considered an aphrodisiac. From the Middle Ages, beetroot was used as a treatment for a variety of conditions, especially those relating to digestion and the blood. Platina recommended taking beetroot with garlic to nullify the effects of 'garlic-breath' (Apicius De Re Coquinaria, Oct 11, 2012). It has been suggested the pigment molecule betanin in the root of red beets may protect against oxidative stress and has been used for this purpose in Europe for centuries (Platina De Honesta Voluptate et Valetudine, Oct 29, 2014). All parts of the beet plant contain oxalic acid.

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Beet greens and Swiss chard are both considered high oxalate foods which have been implicated on the formation of kidney stones. Consuming fruits and vegetables of all kinds has long been associated with a reduced risk of many lifestyle-related health conditions. Many studies have suggested that increasing consumption of plant foods like beetroot decreases the risk of obesity and overall mortality, diabetes, heart disease and promotes a healthy complexion and hair, increased energy, overall lower weight. Heart health and blood pressure: A 2008 study published in Hypertension examined the effects of ingesting 500 mls of beetroot juice in healthy volunteers and found that blood pressure was significantly lowered after ingestion. Researchers hypothesized this was likely due to the high nitrate levels contained in beet juice and that the high nitrate vegetables could prove to be a low cost and effective way to treat cardiovascular conditions and blood pressure. Another study conducted in 2010 found similar results that drinking beetroot juice lowered blood pressure considerably on a dose-dependent basis (Beetroot: Health Benefits, Feb 23, 2017).

MATERIALS AND METHODS

Drugs and chemicals: The standard drug topotecan was used as standard drug and other chemicals used for the extraction

and phytochemical screening were provided by Institutional Store and were of LR and AR grade.

Tumour cells: Ascites sarcoma 180 (S180A) is a transplantable tumour which causes hypocalcaemia in tumour-bearing mice, and stimulates bone resorption without parathyroid hormone-like activity were provided by were provided by the Amala Cancer Research Centre, Thrissur, Kerala, India.

Instruments: Lambda max of vitexin was determined by U. V spectrophotometer. The IR spectra of ME-BRT were recorded by ABB Bomem FT-IR spectrometer MB 104 IR spectra recorder with KBr pellets. The H^1-NMR spectra of synthesized compounds were obtained from Bruker Advanced II 400 MHz spectrometer using TMS as an internal standard in $CDCl_3$/DMSO. All the Mass spectra of ME-BRT were recorded by using HPLC-MS. The IR, H^1-NMR, ^{13}C-NMR and Mass spectra were assigned to elucidate the structure of vitexin present in ME-BRT.

MATERIALS AND METHODS

Chemistry

Extraction methodology (Raj.K. Bansal 5th edition**):** Weigh 20 g of beet root paste (root can be mashed or grinned to prepare a paste) into a 250 ml round-bottomed flask. Add 50 ml of methanol and 60 ml of dichloromethane. Heat the mixture under reflux for 5 min on stem-bath with frequent shaking. Filter the mixture under suction and transfer the filtrate to a separating funnel. Wash this mixture containing bioactive compounds with three portions of 150 ml each with sodium chloride solution. Dry the organic layer over anhydrous magnesium sulphate. Filter and evaporate most of the solvent in vacuum without heating and obtained methanolic extract of beet root (ME-BRT) of Beta vulgaris.

Phytochemical screening (Dandiya and Sharma, 2nd edition; Devala Rao, 2008; Jaswant Kaur, 2010): Preliminary Phytochemical screening of ME-BRT had shown the presence of various bioactive compounds such as carbohydrates, amino acids and peptides, phytosterols, carotenoids, and polyphenols etc.

Spectral characterization of vitexin and isovitexin: Vitexin and isovitexin were characterized from ME-BRT by using the following instruments: U. V spectrophotometer; ABB Bomem FT-IR spectrometer; Bruker NMR spectrometer; HPLC-MS etc.

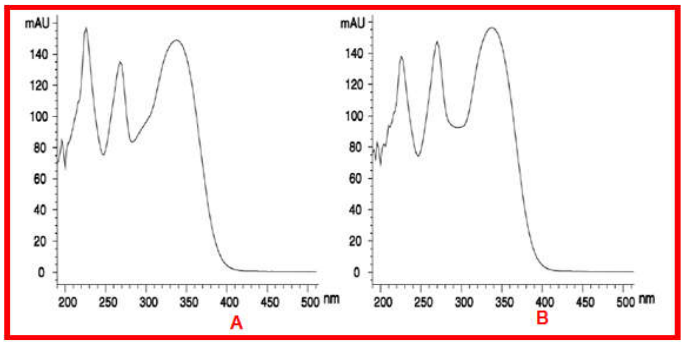

Fig 1. The UV spectra of (A) vitexin; (B) isovitexin

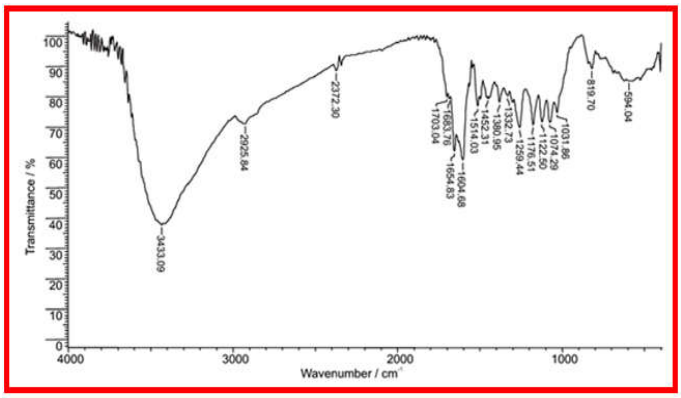

Fig 2. IR spectra of vitexin

Pharmacology

Molecular docking (Pelletier *et al.*, 2005): Molecular docking is defined as an optimization problem, which would describe the "best-fit" orientation of a ligand that binds to a particular protein of interest. During the course of the process, the ligand and the protein adjust their conformation to achieve an overall "best-fit" and this kind of conformational

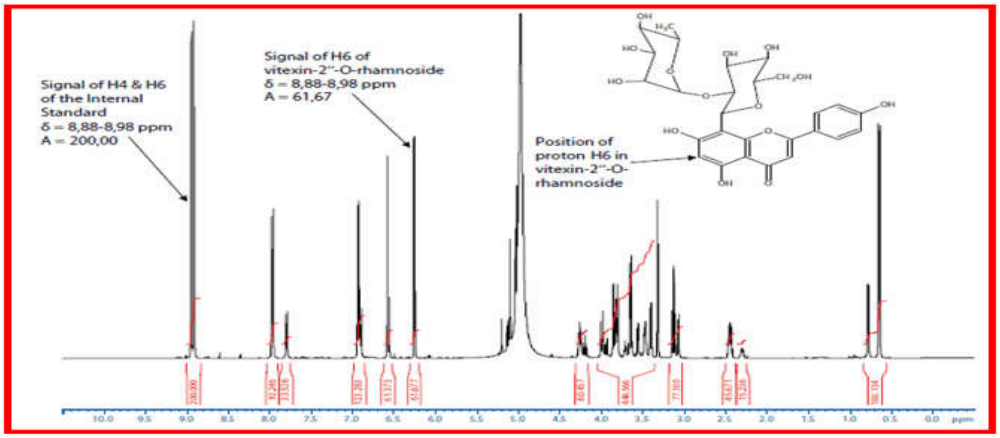

Figure 3. NMR spectrum of vitexin-2"-O-rhamnoside and the Internal Standard 2-hydroxy-3, 5-dinitro benzoic acid. For quantification of the content of the principal component the signals of proton H6 of the vitexin-2"-O-rhamnoside and of the aromatic protons (H4 and H6) of the 2-hydroxy-3, 5-dinitro benzoic acid are used

Table 1. For overall spectral data of vitexin

UV-Vis	
Lambda-max	UV : 335, 271 nm
Extinction coefficient	log ε: 4.08, 4.15
IR	
Major absorption bands	C=O absorption band at 1624 (carbonyl), 3610, 3558 (hydroxyl) 2935, 1149, 1097 (C-H stretch) and 1568, 1501 cm^{-1} (aromatic).
NMR	
Proton NMR	^{1}H-NMR (500 MHz, CD_3COCD_3 + D2O): δ 3.53 (1H, m, H-5″), 3.54 (1H, m, H-3″), 3.57 (1H, m, H-4″), 3.74 (1H, dd, J = 12.3, 5.5 Hz, H-6a″), 3.77 (1H, dd, J = 12.3, 2.0 Hz, H-6b″), 4.11 (1H, t, J = 9.0 Hz, H-2″), 4.85 (1H, d, J = 9.9 Hz, H-1″) 6.44 (1H, s, H-8), 6.60 (1H, s, H-3), 6.95 (2H, d, J = 8.6 Hz, H-3′, 5′), 7.85 (2H, d, J = 8.6 Hz, H-2′, 6′).
Carbon-13 NMR	^{13}C-NMR (125 MHz, CD_3COCD_3 + D2O): δ 61.7 (C-6″), 70.6 (C-4″), 72.0 (C-2″), 74.5 (C-1″), 79.2 (C-3″), 81.6 (C-5″), 95.3 (C-8), 103.4 (C-3), 104.3 (C-10), 108.7 (C-6), 116.7 (C-3′, 5′), 122.4 (C-1′), 129.0 (C-2′, 6′), 157.7 (C-9), 161.0 (C-5), 162.0(C- 4′), 164.5 (C-7), 165.0 (C-2), 183.1 (C-4)
MS	
Masses of main fragments	ESI-MS (M+H)+ m/z 433.1

Table 2. For mass fragmentation

Compounds	m/z	Ions (related normalised abundance (%)
		Vitexin
1.	433.1134	$(M+H)^+$ (44)
2.	314..0798	$(M+H-120)^+$+H: $^{0.2}X^+$+H (11)
3.	313.0719	$(M+H-120)^+$: $^{0.2}X^+$ (53)
4.	284.0865	$^{0.2}X^+$-CHO(100)
5	283.0606	$(M+H-150)^+$: $^{0.2}X^+$ or CH_2O (88)
6.	256.0736	$^{0.2}X^+$ - C_2HO_2 (36)
7.	255.0657	$^{0.2}X^+$ - 2CHO(31)
8.	165.0188	$^{1.0}A^+$ - CH_2O (19)
9.	121.0290	$^{0.2}B^+$ (20)
		Isovitexin
1.	433.1134	$(M+H)^+$ (68)
2.	415.1029	$(M+H-18)^+$+ E_1^+(11)
3.	337.0712	$(M+H-96)^+$: $^{0.4}X^+$-$2H_2O$(12)
4.	314.0798	$(M+H-120)^+$ +H: $^{0.2}X^+$ +H (8)
5	313.0719	$(M+H-120)^+$: $^{0.2}X^+$ (43)
6.	284.0865	$^{0.2}X^+$ -CHO (44)
7.	283.0606	$(M+H-150)^+$: $^{0.2}X^+$ or $^{0.2}X^+$ - CH_2O (100)
8.	256.0736	$^{0.2}X^+$ - C_2HO_2 (9)
9.	255.0657	$^{0.2}X^+$ - 2CHO (12)
10.	165.0188	$^{1.3}A^+$ - CH_2O (7)
11.	121.0290	$^{0.2}B^+$ (16)

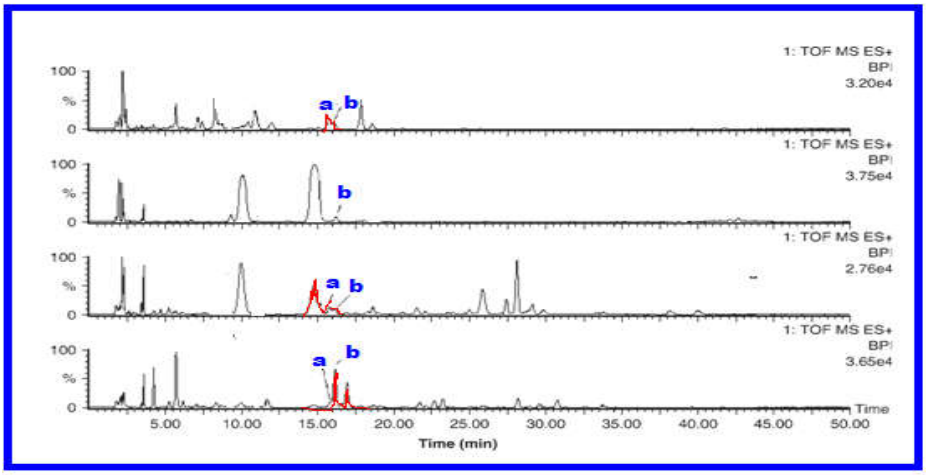

Fig 4. Base peak intensity (BPI) chromatograms of ME-BRT. Key to peaks identification: a = vitexin and b= isovitexin

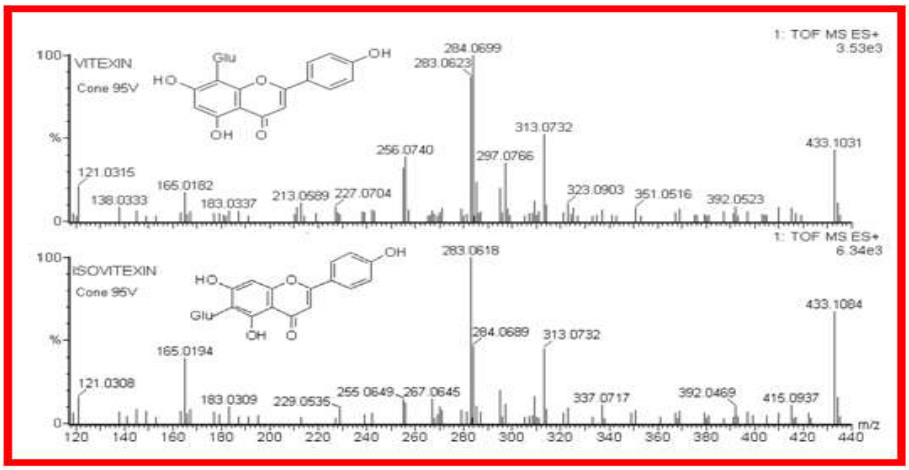

Fig 5. Exact mass in-source CID fragment ion mass spectra at an applied cone voltage of 95 V for vitexin and isovitexin extracted from ME-BRT

adjustment resulting in the overall binding is referred to as "induced fit". Structure based drug design attempts to use the structure of proteins as a basis for designing new ligands by applying accepted principles of molecular recognition. The scoring function was described by a "Master equation" developed by Böhm: $\Delta G_{bind} = RTlnk_d$

Where: k_d = (Receptor)(Acceptor)/(Complex).

$$\Delta G_{bind} = \Delta G_{desolvation} + \Delta G_{motion} + \Delta G_{configuration} + \Delta G_{interaction}$$

Molecular Docking Study: Type of Work: Computational Analysis**.** Job Id: NRS/AS/0044/04/2017**.** PDB Code: 1A36.

Structure of topoisomerase I: Crystalline structure of the target protein Topoisomerase I with PDB id 1A36 was retrieved from protein data bank and protein clean-up process was done and essential missing hydrogen atom were been added. Different orientation of the lead molecules Vitexinwith respect to the target protein was evaluated by Auto dock program and the best dock pose was selected based on the interaction study analysis.

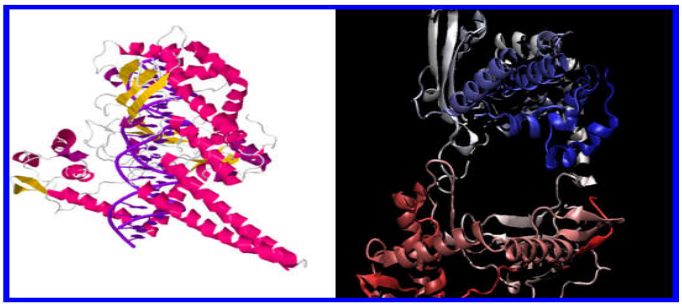

Fig 6. Structure of topoisomerase I

Protocol for the study of acute oral toxicity of ME-BRT: In the present study the acute oral toxicity of the ME-BRT was performed by acute toxic class method (OECD guidelines – 423" for testing of chemicals, 2001). The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) of **CPCSEA: IAEC/XXIX/01/2017.**

Evaluation of in vivo Antitumor Activity of ME-BRT on Sarcoma-180 Ascite (S-180-AC) rat model (Goldin *et al*., 1983; Chashoo *et al*., 2011): This was carried out according to the methods described by Monks *et al*. and Chashoo *et al*. S-180 cells were harvested from the peritoneal cavity of Swiss albino rats, used for propagation, harbouring 8–10-day-old ascetic tumour. On day 0, 1×10^7 cells/animal was injected i. p. into the peritoneal cavity of albino rat. The tumour infected animals were then randomized and divided into five groups (each group contain 6 rats).

A. Group I: Normal Control Group (only normal saline, 0.2 ml / mouse)
B. Group II: T. Control (normal saline, 0.2 ml+S-180-AC, 1×10^7 i. p)
C. Group III: Standard (S-180-AC, 1×10^7 i. p + TOPOTECAN (25 mg/ml inj.).
D. Group IV: ME-BRT (S-180-AC, 1×10^7 i. p + 100 mg/kg orally)
E. Group V: ME-BRT (S-180-AC, 1×10^7 i. p + 200 mg/kg orally).

On day 1, blood collection from retro-orbital plexus was carried out and the blood samples (0.3 ml) in EDTA were used for the assessment of hematological parameters such as haemoglobin (Hb) content, red blood cell (RBC) count, total white blood cell (WBC) count, DLC and platelet count. On day 2, S-180-AC cell suspension was withdrawn from 1.5 ml of PBS solution and the tumour cell count was done by using Neubauer chamber under the light microscope. The PBS was added to make a concentration of 1×10^7 cells in 0.1 ml. For tumour induction in study each experimental animal (Group-II to Group-V) was injected with 1×10^7 S-180-AC cells i.e. 0.2 ml by intra peritoneal route. After 24 h of the tumour cells inoculation, the animals were treated with STD drug and test extracts according to their respective groups once daily for next 10 days. On day 12th, blood collection was done again by retro-orbital puncture for haematological assessment. The animals were followed till death. The parameters for antitumor activity in study were recorded as followed. Determination of the percentage increase in life span (PILS): It is calculated from the mean survival time (MST) values. MST (days) =Total number of days survived by all animals in the group/Number of animals in the group. For each group, Percent increase of lifespan (% ILS) was determined by the following formula: PILS (%) = ((MST of treated group/MST o f control group) −1) × 100. All the animals of each group were sacrificed after a particular day and the ascitic fluid was collected from the peritoneal cavity of each mouse for the evaluation of tumour weight and cell number. The percent inhibition of tumour was calculated as: The percent inhibition of tumour = ((Average number of cells in control - Average number of cells in treated animals) / (Average number of cells in control)) x 100.

RESULTS AND DISCUSSION

Lambda max refers to the wavelength in the absorption spectrum where the absorbance is maximum. Generally molecules absorb in a wavelength range centered on the lambda max. It acts as a single quantitative parameter to compare the absorption range of different molecules. Lambda max of vitexin was determined by U. V spectrophotometer. Lambda max and extinction coefficient of vitexin was found to be 335, 271 nm, log ε: 4.08, 4.15. FT-IR data of ME-BRT showed that the isolated compound presented the characteristic intensities of C=O absorption band at 1624 (carbonyl), 3610, 3558 (hydroxyl) 2935, 1149, 1097 (C-H stretch) and 1568, 1501 cm^{-1} (aromatic). The ^{1} H NMR (400 MHz, d_6 -DMSO) spectrum showed a chelated hydroxyl proton (d 13.18, 1H, s), an aromatic singlet (d 6.28, 1H, s), a downfield signal (d 6.79, 1H, s), suggestive of a flavonoid[8] and an AA'BB' spin system (d 8.03, (2H, d, J=8.5 Hz) and 6.09 (2H, d, J=8.5 Hz)), attributable to a para disubstituted phenyl unit. In addition the ^{1} H NMR spectrum showed a series of signals between d 3.19 and 3.98 (6H), characteristic of a sugar unit. The absence of usual O-glycosidic anomeric proton signal and the presence of signal at d 4.69 (1H, d, J=9.88 Hz) indicated the presence of a C-glycoside. Structure and fragmentation pathway (Fig: 7) of the 6-C-glycosides isovitexin (R=H) annotated according to the nomenclature adopted by Domon and Costello (1988), Li and Clayes (1994) and Ma *et al*. (1997). The superscripts indicate bonds that have been broken. Structure and

fragmentation pathway **(Fig: 8)** for the 8-C-glycosides vitexin (R = H) annotated according to the nomenclature adopted by Domon and Costello (1988), Li and Clayes (1994) and Ma *et al*. (1997). The superscripts indicate bonds that have been broken.

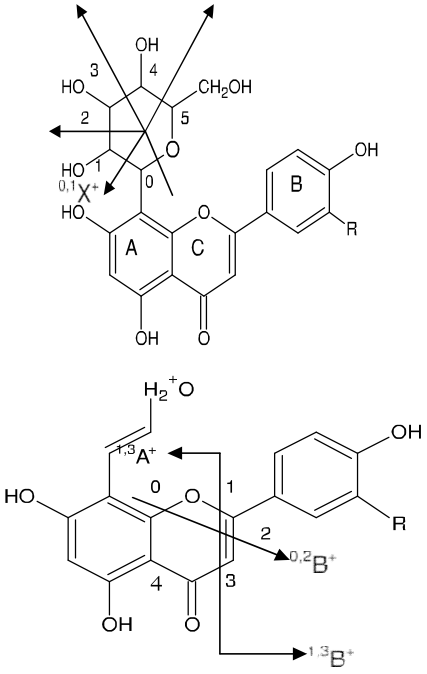

Fig.7. Structure and fragmentation pathway of the 6-C-glycosides isovitexin (R=H).

Molecular Docking

Most of the scoring functions in molecular docking are physics-based molecular mechanics force fields that estimate the energy of the binding pose; a low (negative) energy indicates a stable system and thus a likely binding interaction. Molecular docking is performed to find out the binding affinity or molecular interaction energy (kcal/mol) of docked compounds. Lowest (negative value) energy of docked molecule indicates high binding affinity with the target protein/compound. In silico.molecular docking studies displayed the binding energies: -3.95 k.cal/mol, of vitexin which indicated that the compound had high binding affinity towards the 1A36 -Topoisomerase I in comparison with std. drug topotecan (-2.06 k.cal/mol) (Table 3-6 and Fig 11-12).

Docking results analysis

Table 4. Interaction study

Decomposed Interaction Energies in kcal/mol

hydrogen bonds	polar	cation-pi	other
ARG488 (-0.6153)	ASN722 (-1.8262)	PHE723 (-0.9171)	THR718 (-0.5229)
	LYS532 (-0.7861)		
	LYS587 (-0.7136)		
	ARG590 (-0.4838)		
	HIS632 (-0.3946)		
	LYS443 (-0.3059)		
	ASP533 (-0.2126)		

Acute toxicity study

From the toxicity studies the data revealed that all the synthesized compounds proved to be non toxic at tested dose levels and well tolerated by the experimental animals as there LD_{50} cut of values > 2000 mg/kg b. w. So 100 and 200 mg/ kg. b. w was selected for the evaluation of in vivo pharmacological activity.

Table 3. Vitexin with 1A36 -Topoisomerase I

Results Table

Rank	Est. Free Energy of Binding	Est. Inhibition Constant, Ki	vdW + Hbond + desolv Energy	Electrostatic Energy	Total Intermolec. Energy	Frequency	Interact. Surface
1.	-3.95 kcal/mol	1.28 mM	-3.23 kcal/mol	-0.25 kcal/mol	-3.48 kcal/mol	50%	606.227

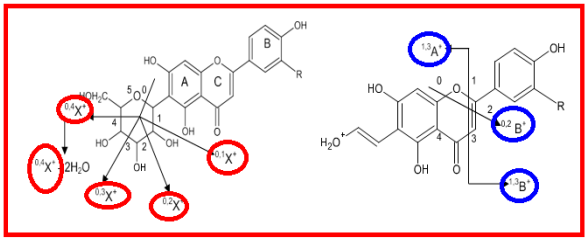

Fig. 8. Structure and fragmentation pathway for the 8-C-glycosides vitexin (R = H)

All the above spectral data assigned the structure of vitexin and isovitexin in ME-BRT.

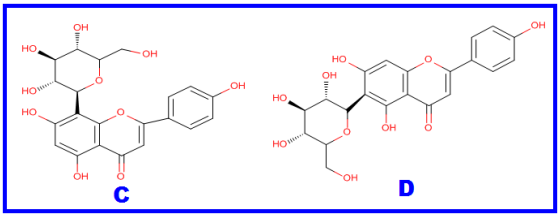

Fig. 9. The structure of (C) vitexin; (D) isovitexin

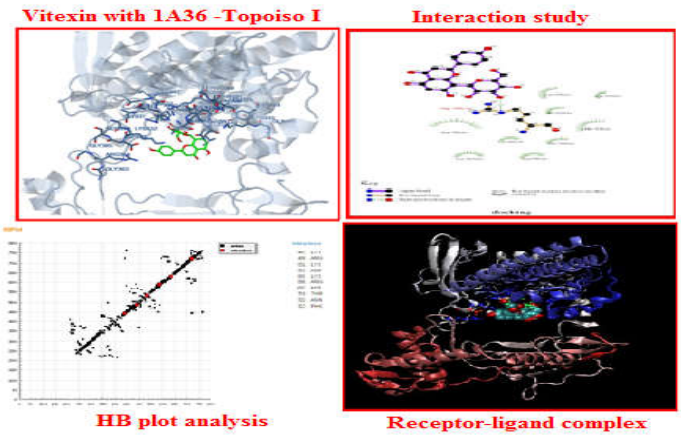

Fig. 10. Molecular docking study report of vitexin

Table 5. Topotecan with 1A36 -Topoisomerase I

Results Table

Rank	Est. Free Energy of Binding	Est. Inhibition Constant, Ki	vdW + Hbond + desolv Energy	Electrostatic Energy	Total Intermolec. Energy	Frequency	Interact. Surface
1.	-2.06 kcal/mol	30.81 mM	-4.38 kcal/mol	+0.40 kcal/mol	-3.98 kcal/mol	50%	602.191

Table 6. Interaction study

Decomposed Interaction Energies in kcal/mol

polar	hydrophobic
ASN745 (-0.5438)	ALA715 (-1.2995)

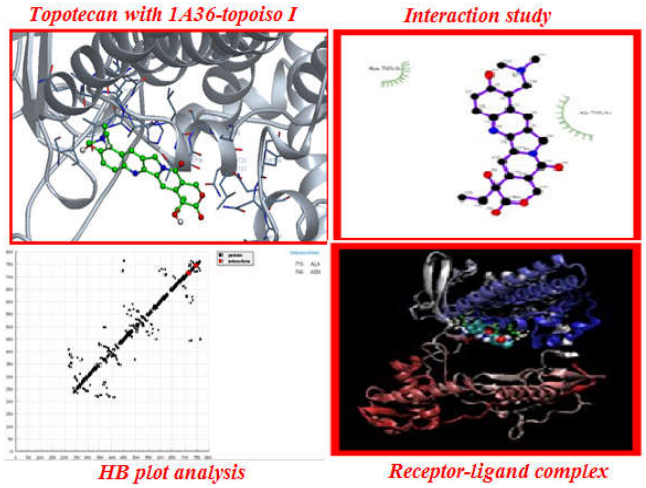

Fig 11. Molecular docking study report of topotecan.

Effect on the haematological parameters: All six animals were in each group. So mortality was less in all groups. The Hb and RBC count were significantly lower in tumour control group compared to normal control group and significantly raise nearly to normal in all treatment groups when compared with control group. The WBC counts were significantly increased in tumour control and it came down to nearly normal range in all treatment groups. The neutrophils were increased and lymphocytes were decreased significantly in tumour control groups and significantly decreased neutrophils and increased lymphocytes in all treatment groups. The platelet count was significantly increased in tumour control ME-BRT (100 mg/kg) ME-BRT (200 mg/kg) group compared to normal group.

Effect on the survival

ME-BRT (100 mg/kg), ME-BRT (200 mg/kg) significantly increased the PILS. While topotican increased the life span of S-180-AC 78.57%, ME-BRT (100 mg/kg) increased it by 57.14% and 71.42 % ME-BRT (200 mg/kg) respectively. So ME-BRT at dose 100 and 200 mg/kg significantly improved the overall survival of all treated animals and Topotecan was not significantly differed from each other in improving the overall survival of S-180-AC.

Inhibition of Tumour growth

The initial screening results for the effects of ME-BRT (100 mg/kg) ME-BRT (200 mg/kg) on Sarcoma-180 ascites model in mice are shown in Table 1.

Both extracts displayed a significant inhibition of tumour growth at doses of 100 and 200 mg/kg with values of 81.90 and 88.33%, respectively, with no mortality, compared standard drug topotecan (95.94%). This shows dose-dependency in its antitumor effect. The ME-BRT displayed the highest tumour growth inhibition at dose 200 mg/kg.

Table 7. A: for the assessment of haematological parameters

Group	Hb (g/dl)	RBC(1×10^6/mm^3)	WBC(1×10^3/mm^3)
I	12.9±0.3813	9.17±0.008165	6.22±0.00732
II	7.075±0.0957	6.163±0.015	7.622±0.002944
III	11.85±0.0577***	8.163±0.00957***	6.149±0.00263**
IV	9.875±0.05*	7.985±0.0173*	6.143±0.05272*
V	11.8±0.1155**	8.165±0.00577***	6.137±0.001414**

Table 7-B: for the assessment of haematological parameters

Group	Neutrophils (%)	Lymphocytes (%)	Platelets (1×10^3/mm^3)
I	13.38±0.2754	87.45±0.3416	3.895±0.002646
II	28.43±0.2217	62.4±0.216	12.17±0.005066
III	12.65±0.1291***	84.55±0.2082***	3.939±0.001708***
IV	11.45±0.1291**	79.73±0.1708**	4.103±0.002082**
V	12.6±0.216***	84.75±0.1291***	3.954±0.004113***

Table 8: for PILS

Group	Total number of days survived by all animals in each group	MST(days)	PILS (%)
II	14	2.725±0.09574	-
III	25	5.475±0.3403	78.57
IV	22	4.475±0.09574	57.14
V	24	4.8±0.08165	71.42

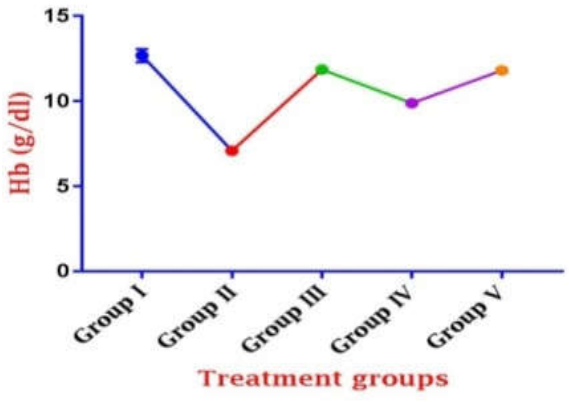

Fig. 12. Comparison of Hb level in different treatment groups received S-180-AC cells

Table 9-A. for inhibition of Tumour growth

Group	B. Wt. Day 1	B. Wt. Day 5	B. Wt. Day 9	B. Wt. Day 12	Wt. of tumour (gm)
I	20.07±09845	20.13±0.0954	20.15±0.05774	20.25±0.05774	-
II	22.22±0.01291	23.83±0.09574	27.15±0.05774	29.72±0.01826	8..725±0.005774
III	21.75±0.01414	21.13±0.008165*	19.2±0.8165**	17.92±0.005774**	0.485±0.005774**
IV	21.75±0.01708	21.19±0.08965*	21.4±0.08165*	21.60±0.009574**	3.275±0.00577**
V	21.75±0.01291	20.13±0.09345**	19.12±0.005***	19.05±0.05774***	2.738±0.00957***

Table 9-B. for inhibition of Tumour growth

Group	Dose mg/kg	Volume of ascetic fluid (ml)	No. of tumour cells ($1x10^7$)	% of tumour growth inhibition (TGI)	Mortality %
II	S-180-AC (1×10^7 cell)	8.89	686.75	0.00	0
III	Topotecan (25 mg/ml)	0.49	13.99	95.94	0
IV	ME-BRT (100 mg/kg)	3.15	110.22	81.90	0
V	ME-BRT(200 mg/kg)	2.81	73.05	88.33	0

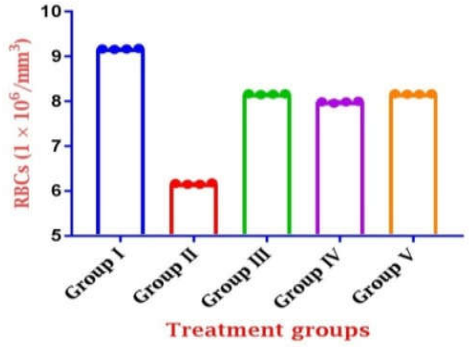

Fig. 13. Comparison of RBCs count in different treatment groups received S-180-AC cells

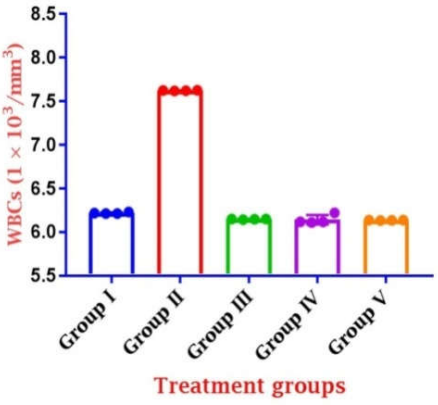

Fig. 14. Comparison of WBCs count in different treatment groups received S-180-AC cells

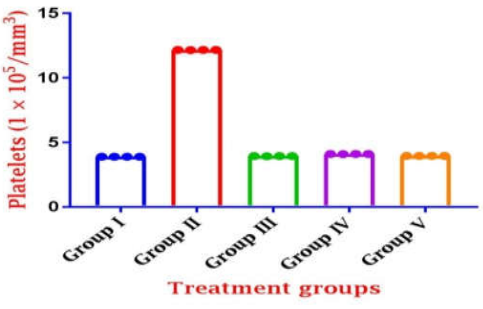

Fig 15: Comparison of plateltes count in different treatment groups received S-180-AC cells

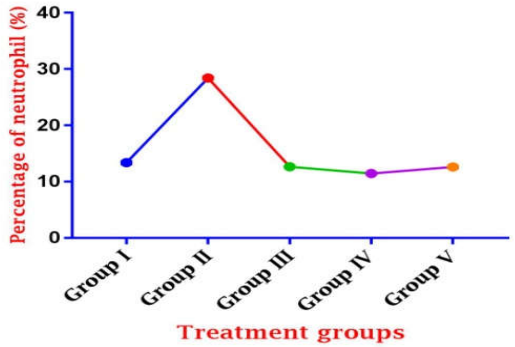

Fig 16. Comparison of % of neutrophil in different treatment groups received S-180-AC cells

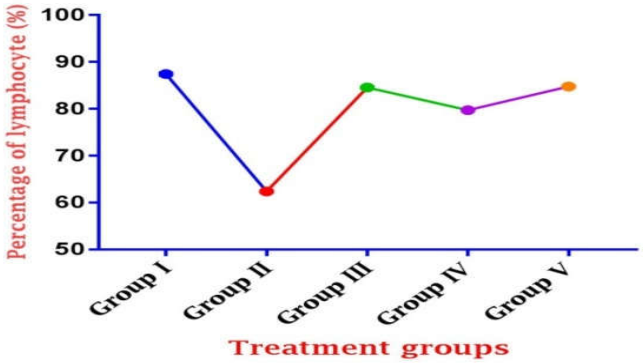

Fig 17. Comparison of % of lymphocytes in different treatment groups received S-180-AC cells

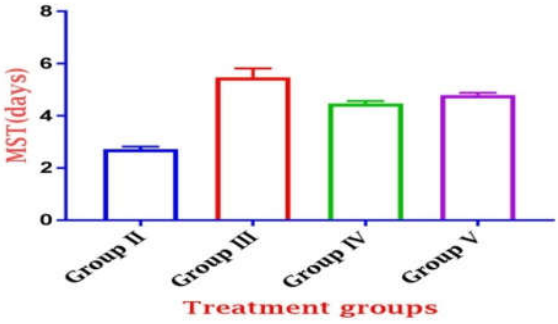

Fig 18. Comparison of MST in different treatment groups received S-180-AC cells

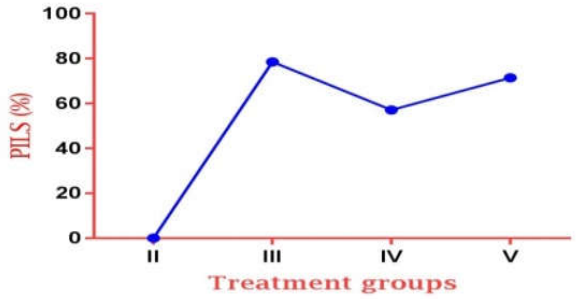

Fig 19. Comparison of PILS in different treatment groups received S-180-AC cells

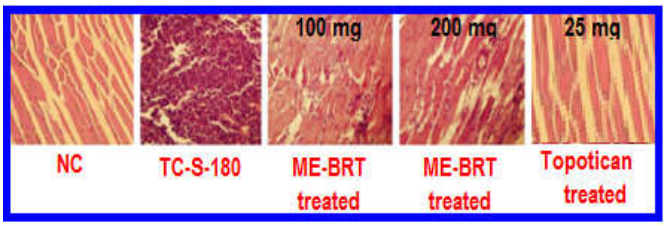

Fig .20. Tumour growth inhibition

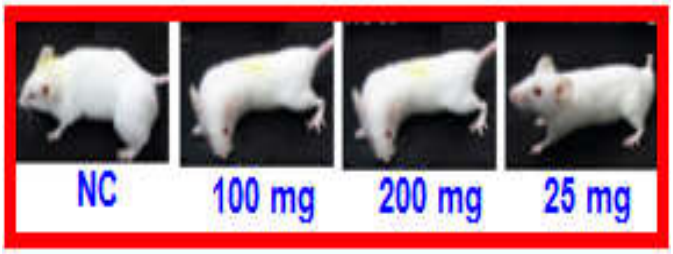

Fig. 21. Tumour growth inhibition by extracts and std. Drug

Conclusion

From the present experimental data here we concluded that the methanolic extracts of beet root (ME-BRT) showed the presence of bioactive molecules vitexin and isovitexin which were characterized by modern analytical techniques such as IR, ^{1}H-NMR, ^{13}C-NMR and HPLC-MS spectrometry and had the potential ability to inhibit the growth of tumour induced by SARCOMA-180 ascites on rat model and in silico molecular docking results displayed that vitexin had the higher affinity to bind with topoisomerase-I enzyme in comparison to standard drug topotecan and act as potential inhibitor of topoisomerase-I.

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