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Research Article

Metabolite Profiling, *In-vitro* Anticancer Activity of *Jatropha heynei* Followed by Molecular Docking Studies

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ABSTRACT

Jatropha heynei of family Euphorbiaceae is an endemic herb with ethno-medicinal values. The present study investigates the phytochemical profiling, *in-vitro* anticancer activity against Dalton's lymphoma ascites (DLA) and ehrlich's ascites carcinoma (EAC) cell lines and *in-silico* molecular docking analysis of isolated chemicals of *J. heynei* tuber ethanolic extract. High resolution liquid chromatograph mass spectrometer (HR-LCMS) analysis of ethanolic tuber extract revealed the presence of 19 prominent phytochemical compounds. Six compounds meptazinol, 8-amino-7-oxononanoate, dihydrodeoxystreptomycin, albendazol (II), hydroxytinidazole, azoprocarbazine were present in high quantity, of which azoprocarbazine was shown with anticancer activity. There were several compounds with a number of other therapeutic properties. The ethanolic extract of *J. heynei* tuber showed significant *in-vitro* cytotoxic activity against DLA (IC_{50}=64.14 µg mL^{-1}) and EAC (IC_{50}=71.42 µg mL^{-1}) cancer cell lines, almost comparable to the value of standard curcumin (IC_{50}=54.36 µg mL^{-1}). Molecular docking and *in-silico* analysis of the compounds such as azoprocarbazine, ecgonine and meptazinol displayed strong interactions with the anticancer target proteins such as vascular endothelial growth factor (VEGF), tyrosine kinase (TK), and matrix metalloproteins (MMP) when compared with their respective drug inhibitors pazopanib, imatinib and batimastat. Compounds with wide range of biological activities suggested that *J. heynei* could be exploited for formulating new drugs in the treatment of several diseases and disorders.

Introduction

In many developing countries, lots of people get their health care from traditional practitioners and plants in order to meet health benefits. Even though modern medicines can be used along with these traditional methods, herbal medicines have often stayed popular because of their history and culture. The assessment of various plant products based on their traditional medicinal uses and therapeutic potential leads to the development of newer and more new drugs for treating a variety of ailments. This fact serves as the foundation for the creation of novel drugs derived from various plant sources. *Jatropha heynei* N. P. Balakr., a rare and endemic herb belonging to the spurge family Euphorbiaceae, grows in the nutrient rich well-drained soil found in Southern parts of India. *Jatropha* species finds application in traditional medicine in Africa, Asia, and Latin America to cure various ailments; some are ornamental plants and energy crops.[1] In the southern part of India *J. heynei* is used medicinally to treat burns and cut wounds[2] and the local folklore of Chitradurga, Karnataka use tubers to treat arthritis and also for wound healing. The extracts and compounds isolated from the *Jatropha* species have been shown to have cytotoxic, antimicrobial, anti-inflammatory, antioxidant, insecticidal, and larvicidal properties.[3]

Cancer is one of the high risk non-communicable disease in human health and is often a tough challenge to modern medicine. It is considered as the second deadliest disease all over the world, is a major cause of reported human deaths worldwide, with approximately nine million deaths and more than 14 million new cases reported each year.[4] Deep understanding of the mechanisms of formation

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and spread of tumor is important in the development of effective therapeutic agents to induce apoptosis. Apoptosis is a physiological process of cell death, which is well-regulated; thus, cellular inflammatory responses are not induced, making it a safer and better feature of a therapeutic candidate.[5] Inhibition of angiogenesis, tumor vascularization and tyrosine kinase (TK) activity are key therapeutic points that prevent metastasis and cause apoptosis.[4] Thus, TK, vascular endothelial growth factor (VEGF), and matrix metalloproteinases (MMP) are important anticancer target receptors. Plants are exceptional and dependable sources of novel anticancer therapeutics accounting for more than 60% of the various anticancer agents available.[6] The medicinal value of higher plants lies in some chemically active substances that produce a definite physiological action on the human body.[7] Campothecin, vinblastine, vincristine, etoposide, teniposide, and paclitaxel some are examples of plant-derived anticancer agents.[8,9]

Currently, many gaps exist in our knowledge of interaction of compounds with target proteins and experimentally testing all the possible interactions is not feasible. Recent advances and developments of system pharmacology and computational (*in-silico*) approaches provide powerful tools for exploring the polypharmacological profiles of natural products.[10] The rational drug design in combination with structure-based modelling and rapid screening methods offer significant potential for identifying and developing lead anticancer molecules. Thus, the molecular docking method plays an important role in screening a large set of molecules based on their free binding energies and proposes structural hypotheses to study the inhibition of target molecules. *In silico* approaches, molecular docking and molecular dynamic simulations are being broadly used in computational drug discovery identification of novel compounds.[11]

A perusal of literature indicated that a number of medicinal plants have been studied over the years for the presence of anticancer compounds. However, several other medicinal plants are still underexplored or not studied for the anticancer activity. Although *J. heynei* has been studied for some of its cytotoxic activities,[12] a detailed systematic study of compounds for the above activity in the plant species has not been undertaken. This promoted the authors to characterize compounds with anticancer activity and to determine the *in-silico* molecular docking of host plant metabolites in relation to receptors.

Materials And Methods

Collection of Plant Material and Preparation of Plant Extract

J. heynei plant specimens were collected from Surammanahalli village: (14.6262º N 76.608593ºE) of Chitradurga district, Karnataka, India. *J. heynei* was identified based on morphological characteristics.[13] The typical herbarium specimen was collected and maintained in the herbarium Centre in the Department of Applied Botany, Kuvempu University (Voucher No-KU/AB/05). Apparently, healthy tuber samples were collected in sterile polythene bags and processed in the laboratory within 24 hours. The plant samples were washed under running tap water to remove soil adherents and shade-dried for 20–25 days in sterile conditions and then finely powdered (particle size 66–1055 µm) using the grinder. Samples were subjected to soxhlet extraction using ethanol solvent.[14] The crude extract was filtered and dried in vacuum, under ambient conditions. The filtrate was re-extracted in ethanol and then in DMSO (10% Dimethyl Sulphoxide, Himedia Laboratories Pvt.Ltd) and stored at 5°C until used.

Preliminary Phytochemical Analysis

The ethanolic crude extract of tuber was subjected to qualitative phytochemical screening for the presence of secondary metabolites by the standard procedures.[15]

Cytotoxicity Assay *In-vitro*

Cytotoxicity of ethanolic tuber extract was assessed by trypan blue exclusion method using Dalton's lymphoma ascites (DLA) and ehrlich's ascites carcinoma (EAC) cell lines obtained from Amala Cancer Research Centre Thrissur, Kerala, India. The tumor cells, aspirated from the peritoneal cavity of tumor-bearing mice, were washed thrice with BPS or normal saline, and cell viability was determined by the trypan blue exclusion method.[16,17] Viable cell suspension (1×10^6, 0.1 mL^{-1}) was added to tubes containing various concentrations of the extract and the volume was made up to 1-mL using phosphate buffered saline (PBS, 0.2 M, pH 7.4). Control set contained only the cell suspension. These assay mixtures were incubated at 37°C for three hours. Further, the cell suspension was mixed with 0.1-mL of 1% trypan blue stain and kept for 2–3 minutes and loaded on a hemocytometer. Cells that take up the trypan blue stain are considered as dead while unstained cells were live, that were counted separately. The percentage cytotoxicity was calculated as follows:

$$\text{Cytotoxicity (\%)} = \frac{\text{Number of dead cells} \times 100}{\text{Number of live cells + Number of dead cells}}$$

High Resolution Liquid Chromatography Coupled to Mass Spectroscopy Analysis

The high resolution liquid chromatograph mass spectrometer (HR-LCMS) analysis was performed using an Agilent Q-TOF system equipped with dual AJS ESI (electrospray ionization) source at IIT, Bombay. The gradient elution at a flow rate of 0.03 $mL.min^{-1}$ was operated for 30 minutes stop time. The full scan mass spectra were obtained within the range of m/z. 120-1000 amu at 1.00 scan rate. Solvent composition in channels A and B was water (95%) and acetonitrile (5%), respectively. Value switch time 1 was enabled with 5.00 µL injection

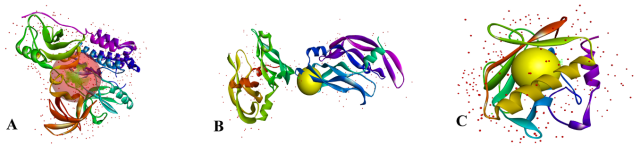


Fig. 1: The 3D crystal structure of human (A) VEGF (1VPF), (B) TK (1FMK), and (C) MMP (2OXU) with their binding sites

volume. The analytical data were optimized using the software system Analyst version: 1.4.2 with background subtraction technique of chromatography. The principle of this technique is to reduce the background problems such as fault peaks and noise.

In-silico Molecular Docking Study

Softwares and Tools Used

Chem Sketch 12.01, Auto Dock 4.2, Discovery Studio v 20.1.0, Auto Dock Vina 1.1.2, Protein Plus, Pre-ADMET, MGL tools, Open Babel, Protein Data Bank (PDB), PubChem, PyMOL, and Swiss ADME.

Ligand Preparation

The compounds-meptazinol (PubChem CID 41049), azoprocarbazine (PandubChem CID 75230) and ecgonine (PubChem CID 91460) reported HR-LCMS analysis of an ethanolic extract of *J. heynei* tuber was used in the present study. The structures of identified compounds were generated using chemdraw and used as ligands.[18] The SD files were converted to their corresponding three-dimensional (3D) structures and saved in pdb format using Open Babel.[19] Water molecules were removed, gasteiger charges were added and non-polar hydrogens were merged using Auto dock software.[20]

In-silico Analysis of Drug-Likeness

The drug-likeness of selected ligand molecules was calculated using swiss ADME.[21] The ligands were subjected to Lipinski,[22] Ghose,[23] Veber,[24] Egan,[25] and Muegge[26] screening. Only those ligands that could satisfy these variants without default were used for docking simulation.

Protein Preparation

The 3D crystal structures of target proteins such as human VEGF, human TK and human MMP were retrieved from Research Collaboratory for Structural Bioinformatics (RCSB) protein data bank (PDB) as 1VPF (crystal structure and functional mapping of the kinase domain receptor binding site), 1FMK (The structure of a large fragment of the c-Src tyrosine kinase, comprising the regulatory and kinase domains and the carboxy-terminal tall) and 2OXU (uninhibited form of human MMP-12) as their respective PDB codes without any complexed ligands (Fig. 1). The protein structures were cleaned, water molecules removed, Kollman charges computed and polar hydrogen added using Auto Dock 4.2 software.[20]

Active Site Prediction

The possible active binding sites of proteins were obtained using DoGSiteScorer.[27] The binding sites with the best volume, surface area, and drug ability score were selected for this study. DoGSiteScorer is a grid-based method, which uses a difference of Gaussian filter to detect potential binding pockets, solely based on the 3D structure of the protein, and splits them into sub pockets.

Docking Analysis

Auto Dock 4.2 was used to transform both receptor and ligand structures to pdbqt file format, which includes atomic charges, atom-type definitions, and for ligands, topological information (rotatable bonds) with a grid centered to ensure coverage of the binding site of the structure. Auto Dock Vina was used to perform docking simulations, generating 9 conformations of ligand in complex with the receptor, which were finally ranked based on binding energy.[28] The genetic algorithm (GA) parameters, which guided the docking procedure, were set as 200 (population size), 70 (generations), and 3 (number of solutions). Bond energies, such as hydrogen bond (Hb), van der Waals (VdW), and electrostatic interaction that occurred between the protein ligands were identified. The resulting conformations were visualized in the Discovery Studio Visualizer.[29]

Absorption, Distribution, Metabolism, Excretion and Toxicity Properties (ADMET) Analysis

The *in-silico* ADMET properties of the selected compounds were calculated as an alternative approach to the expensive experimental evaluation of ADMET profiles. For ADMET assessment, we used Pre-ADMET[30] and Swiss ADME[21] to examine the different pharmaco-kinetic parameters of the docked ligands.

Results And Discussion

Qualitative Phytochemical Analysis

To explore the pharmaceutical value of any medicinal plant, one of the initial steps is to screen the plant for its phytochemicals, as it gives an idea of the compounds present in it. The ethanolic crude extract of *J. heynei* tuber contained alkaloids, phytosterols, saponins, phenolic compounds, flavonoids and steroids (Table 1). The presence of secondary metabolites like phenolic compounds, flavonoids and alkaloids in the tuber extract indicated their involvement in the diverse biological activities, mainly anti-inflammatory, antimicrobial, antioxidant, cytotoxic and antitumor activities.[31-33]

In-vitro Cytotoxicity of Ethanolic Extract Against DLA and EAC Cells

In-vitro cytotoxicity of *J. heynei* ethanolic extract showed dose-dependent cytotoxic activity in all tested concentrations against DLA and EAC cancer cells, and

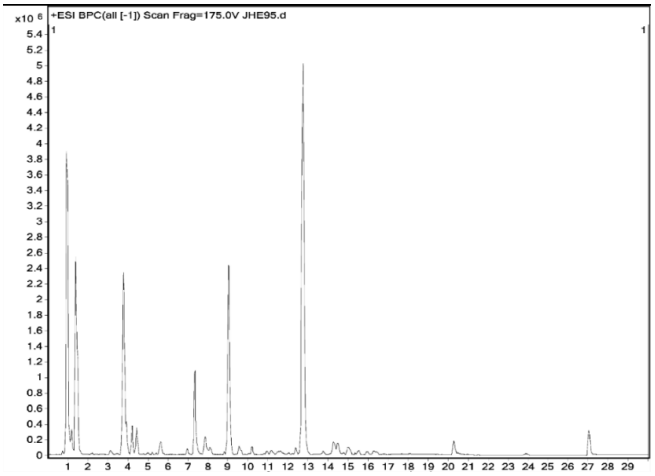


Fig. 2: HR-LCMS Chromatogram of ethanol extract of *J.heynei* showing prominent compounds corresponding to retention time

Table 1: Phytochemical constituents* in the ethanol tuber extract of *J. heynei*

S. No	*Phytochemicals*	*Present/Absent*
01	Alkaloids	+
02	Phytosterols	+
03	Saponins	+
04	Phenolic compounds	+
05	Tannins	-
06	Flavonoids	+
07	Terpenoids	-
08	Cardiac glycosides	-
09	Steroids	+

Note:* Qualitative phytochemical analysis of tuber extract done by the method of Kokate *et al.*, 1997. + =Present. - = Absent.

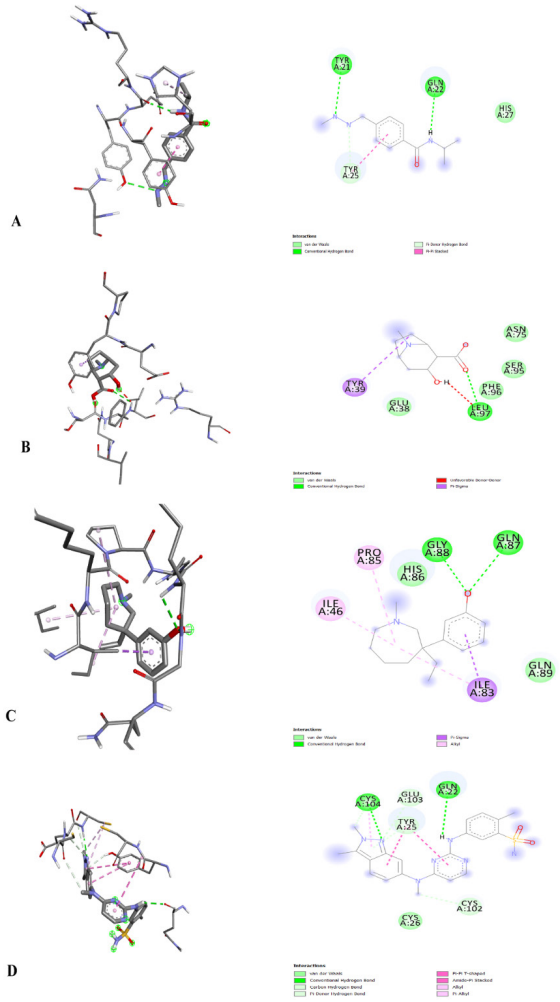


Fig. 3: The 2D and 3D views of (A) azoprocarbazine, (B) meptazinol (C) ecgonine and (D) pazopanib ligand interactions with VEGF.

particularly the extract was more toxic to the DLA than EAC cells (Table 2). The extract was cytotoxic to DLA cells at the concentration of 200 µg.mL^{-1} and caused 100% cell death. The IC_{50} value (concentration that causes a reduction in cell viability to 50%) of the crude extract against DLA cells was 64.14 µg.mL^{-1} which is almost equal to the IC_{50} value of standard curcumin 54.36 µg.mL^{-1}. While, the cytotoxic effect of the ethanolic extract against EAC cells was 82.05% at the concentration of 200 µg.mL^{-1} of crude extract used and the IC_{50} value of extract against EAC cells was 71.42 µg.mL^{-1} when compared to the IC_{50} value of standard curcumin 54.36 µg.mL^{-1}. The significant *in-vitro* cytotoxic effect of the ethanol extract of *J. heyeni* could be due to the presence of the anticancer compound azoprocarbzine[34] and anti-inflammatory

Table 2: *In-vitro* cytotoxicity of tuber extract of *J. heynei* against DLA and EAC cells

S. No	*Concentration (µgml^{-1})*	*DLA cells*		*EAC cells*		*Standard (Curcumin) Cytotoxicity (%)*	*IC_{50} value Of Standard (Curcumin)*
		Tuber ethanolic extract		*Tuber ethanolic extract*			
		Cytotoxicity (%)	*IC_{50} (µg.mL^{-1})*	*Cytotoxicity (%)*	*IC_{50} (µg.mL^{-1})*		
1	10	30		30		23.15	
2	20	38.12		36.19		34.43	
3	50	58.12	64.18	52.11	71.42	100.05	54.36
4	100	70.05		66.19		100.05	
5	200	100.17		82.05		100.05	

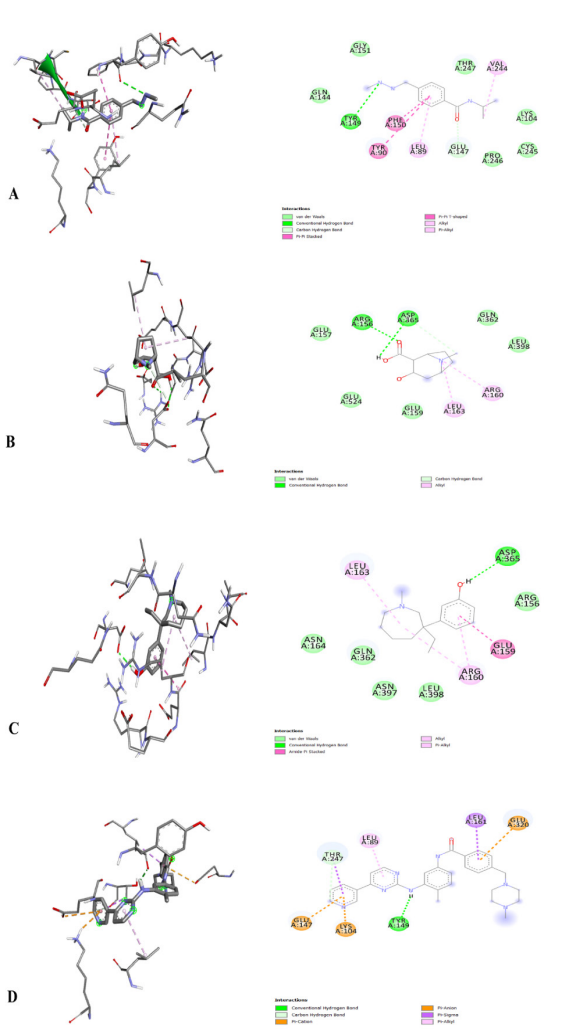


Fig. 4: The 2D and 3D views of (A) azoprocarbazine, (B) meptazinol (C) ecgonine and (D) Imatinib ligand interactions with TK.

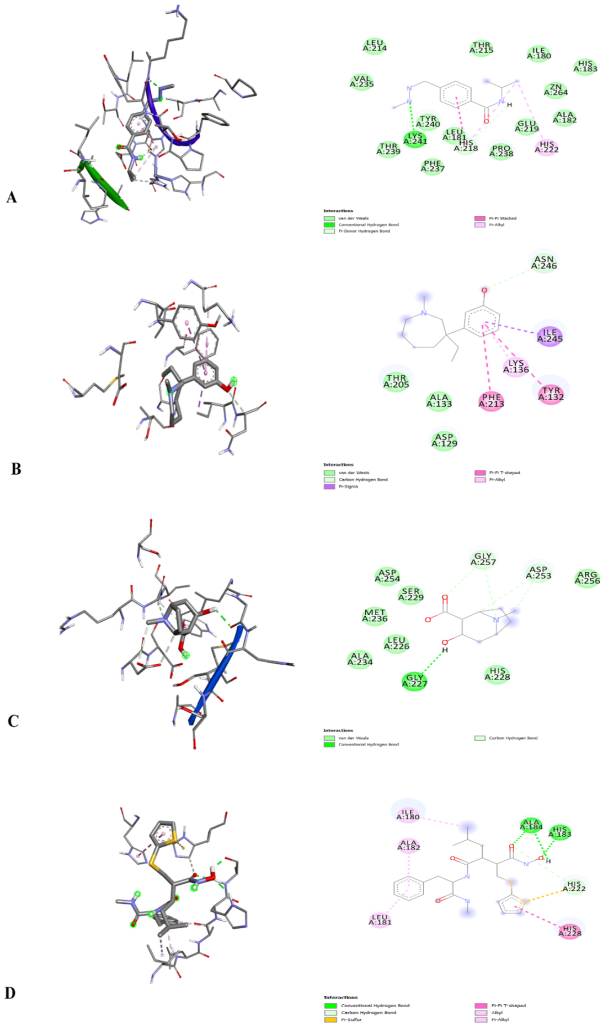


Fig. 5: The 2D and 3D views of (A) azoprocarbazine, (B) meptazinol (C) ecgonine and (D) Batimastat ligand interactions with MMP.

compounds ecgonine[35] and meptazinol.[36] Several studies demonstrated that anti-inflammatory agents could increase apoptosis and sensitivity to conventional therapies and decrease invasion and metastasis making them useful candidates for cancer therapy.[37]

HR-LCMS Phytochemical Analysis

The phytochemical screening of ethanolic tuber extract by HR-LCMS technique resulted in the detection of 19 known chemical compounds and seven unknown compounds (Fig. 2).

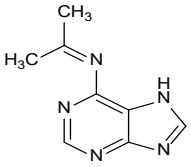
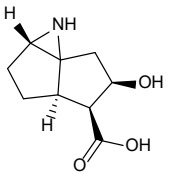
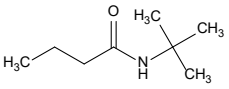
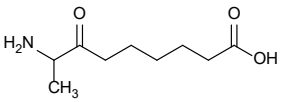
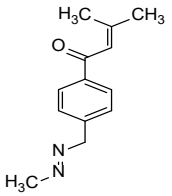
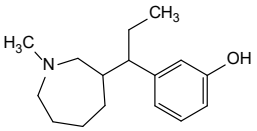
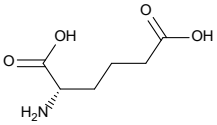
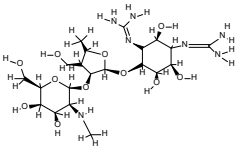
Among them ecgonine, dexpanthenol and meptazinol have anti-inflammatory properties.[35-39] 8-amino-7-oxononanoate, hydroxytinidazole and dihydrodeoxystreptomycin exhibit antimicrobial property.[40-42] Azoprocarbazine is anticancerous,[34] while albendazole II is anti-helminthic.[43] Hydroxytinidazole is both antimicrobial and antiprotozoal.[41] N-isopentenyladenine is a cytokinin, with growth regulatory property (Table 3).

Apart from these compounds, dihydrodeoxystreptomycin, a glycoside derivative of streptomycin is shown with antimicrobial property.[42] Hydroxyltinidazole a derivative of tinidazole has been widely used throughout European countries and developing countries for the treatment of amoebic and parasitic infections.[41] Another compound dexapanthenol is known for its anti-inflammatory property and is popular in treating various skin disorders.[38,39] Albendazole is a standard anti-helminthic drug with broad spectrum of activity against a wide range of helminth parasites.[43] The compound 8-amino-7-oxononanoate, a phosphate-dependent enzyme that helps in biotin synthesis, is antimicrobial.[40] The present study revealed the presence of several compounds with various biological activities in the extract of *J. heyeni*.

Drug-Likeness Results

To determine drug-likeness and physico-chemical properties, compounds in the extract were subjected to the

Table 3: Physico-chemical and biological properties of compounds detected in the Ethanolic extract of *J. heynei* through HR-LCMS technique.

Name of the compound	*RT (min)/mass*	*Molecular formula*	*Molecular structure*	*Biological activities*	*Reference*
N-Isopentenyladenine	0.963/203.1213	$C_{10}H_{13}N_5$		Cytokinin, Growth regulatory	[52]
Ecgonine	0.97/185.1098	$C_9H_{15}NO_3$		Tropane alkaloid, Anti-inflammatory, Immunoregulatory.	[35]
Dexpanthenol	0.981/205.1325	$C_9H_{19}NO$		Anti-inflammatory	[38-39]
N-isopropylterephthaldehyde	0.983/191.0993	$C_{11}H_{13}NO_2$	-	Unknown	
8-amino-7-oxononanoate	0.993/187.1252	$C_9H_{17}NO_3$		Herbicidal, Antimicrobial	[40]
Citrullinen-butyl ester	1.017/231.1535	$C_{10}H_{21}N_3O_3$	-	Unknown	
Azoprocarbazine	1.073/219.136	$C_{12}H_{17}N_3O$		Anticancer	[34]
Meptazinol	1.441/233.1772	$C_{15}H_{23}NO$		Anti-inflammatory, Analgesic.	[36]
L-2-aminoadipic acid	3.858/161.0683	$C_6H_{11}NO_4$		Role as a *Escherichia coli* metabolite and human metabolite	PubChem CID : 92136
Lys Ile	3.984/259.1915	$C_{12}H_{25}N_3O_3$	-	Unknown	
Furegrelate	4.434/253.0692	$C_{15}H_{11}NO_3$	-	Unknown	
5beta-cholestane-3-alpha,7alpha,12alpha,25,26-pentol	5.607/452.3496	$C_{27}H_{48}O_5$	-	Bile alcohol	[51]
Hydroxytinidazole	7.85/ 263.0597	$C_8H_{13}N_3O_5S$		Antiprotozoal, Antimicrobial	[41]
dihydrodeoxystreptomycin	9.018/567.285	$C_{21}H_{41}N_7O_{11}$		Antimicrobial activity	[42]
Meta glu	10.245/278.0943	$C_{10}H_{18}N_2O_5S$	-	Expected metabolite	PubChem: 14843111

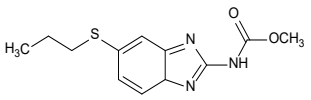
Albendazole II	12.991/313.0737	$C_{12}H_{15}N_3O_5S$		Anthelminthic	[43]
Metaproterenol 3-O-sulphate	12.765/291.077	$C_{11}H_{17}O_6S$	-	Unknown	
Phe leu	14.995/ 78.1604	$C_{15}H_{22}N_2O_3$	-	Unknown	
DL-PDMP	20.283/390.2935	$C_{23}H_{38}N_2O_3$	-	Unknown	

Table 4: Drug-likeness violation of compounds isolated from tuber extract of *J. heynei*

Compounds	*Number of violations*				
	Lipinski	*Ghose*	*Veber*	*Egan*	*Muegge*
Azoprocarbazine	-	-	-	-	-
Ecgonine	-	1	-	-	1
Meptazinol	-	-	-	-	-

Lipinski rule of 5 (RO5), Ghose filter, Veber rule, Egan rule and Muegge rule. Properties such as the number of hydrogen bond donors (HBD), hydrogen bond acceptors (HBA), molecular mass, log P, molar refractivity, rotatable bonds, topological polar surface area (TPSA) and number of rotatable bonds were taken for determining drug-likeness.[22-26] The selected phytochemicals-azoprocarbazine, ecgonine and meptazinol passed all the drug-likeness tests without any default. Ecgonine had defaults in all variants except for Ghose and Muegge test of drug-likeness (Table 4). Compounds whose properties successfully pass these variants were considered to possess good pharmaco-kinetic properties and thus were further subjected to *in-silico* technique such as molecular docking.[44]

Table 5: Molecular docking analysis using Auto Dock Vina for isolated compounds showing estimated binding affinity and interacting residues in the binding sites of VEGF, TK and MMP.

Proteins	Ligand	Binding affinity (kcal/mol)	HB-IR	VDW-IR	PI-IR
VEGF (1vpf)	Azoprocarbazine	-4.8	TYR21, GLN22	HIS27	TYR25, GLN22
	Meptazinol	-4.9	GLY88, GLN87	HIS86, GLN89	PRO85, ILE46
	Ecgonine	-4.2	LEU97	GLU38, PHEA96, SER95, ASN75	-
	Pazopanib	-6.1	GLN22, CYS104	CYS26, GLU103	CYS104
TK (1fmk)	Azoprocarbazine	-6.4	TYR149	GLY151, GLN144, THR247, LYS104, PRO246, LYS245	LEU89
	Meptazinol	-7.0	ASP365	ASN164, GLN362, ASN397, LEU398, ARG156	ARG160
	Ecgonine	-5.5	ARG156, ASP365	GLU157, GLU524, GLU159, GLN362, LEU398	LEU163, ARG160
	Imatinib	-10.6	TYR149	-	LEU89
MMP (2oxu)	Azoprocarbazine	-7.8	LYS241	LEU214, VAL235, THR239, PHE237, TYR240, LEU181, PRO238, GLU219, ALA182, HIS183, ILE180, THR215, PHE237, ZN264	HIS222
	Meptazinol	-6.0	-	THR205, ALA133, ASP129, ASN246	LYS136
	Ecgonine	-5.3	GLY227	ASP254, SER229, MET236	-
	Batimastat	-6.9	ALA184, HIS183	-	LEU181, ALA 182

Note: HB-IR= hydrogen bond interacting residues, MMP=matrix metalloproteins, TK=tyrosine kinase, VEGF= vascular endothelial growth factor, VdW= vanderwaals interacting residues, PI-IR= Pi bonds interacting residues.

Table 6: Physico-chemical properties of compounds in the tuber extract of *J. heynei*

Compounds	*Molecular weight (g mol^{-1})*	*Number of Hydrogen bond acceptors*	*Number of Hydrogen bond donors*	*Number of rotatable bonds*	*Molar refractivity*	*TPSA (A^2)*
Azoprocarbazine	219.2	3	1	5	63.68	53.82
Meptazinol	233.35	2	1	2	76.47	23.47
Ecgonine	185.22	4	2	1	50.89	60.77

Note: TPSA= Total polar surface area

Table 7: Predicted absorption profile of compounds isolated from tubers of *J. heynei*

Compounds	*Log P*	*Log S*	*GI absorption*	*BBB permeability*	*P-gp Substrate*	*Log Kp (cm/s)*
Azoprocarbazine	2.81	-1.76	High	+	No	-6.94
Meptazinol	2.74	-3.56	High	+	No	-5.31
Ecgonine	1.48	0.21	High	-	No	-8.71

Note: Log P= lypophilicity, Log S= solubility, GI= gastro-intestinal absorption, BBB= blood brain barrier penetrability, P-gp= glycoprotein, Log Kp= skin permeability.

Table 8: Predicted molecular toxicity profile of compounds isolated from tubers of *J. heynei*

Compounds	*Ames toxicity*				*Carcinogenicity*		*hERG inhibition*
	TA100_10RLI	*TA100_NA*	*TA153_10RLI*	*TA153_NA*	*Carcino rat*	*Carcino mouse*	
Azoprocarbazine	+	+	+	+	-	-	Low risk
Meptazinol	-	-	-	-	-	-	Low risk
Ecgonine	-	-	+	-	-	+	Low risk

Note: hERG =human Ether-a-go-go gene

Docking Simulations

Molecular docking is an important *in-silico* technique which predicts the mode of interaction between a small ligand and target protein for an established binding site.[45] A compound with a low binding energy is preferred as a possible drug candidate.[46] The binding affinity of three isolated compounds, as simulated by Auto Dock Vina, ranged from -4.2 to -4.9, -5.5 to -7.0, and -5.3 to -7.8 kcal. mol^{-1} for VEGF, TK, and MMP, respectively (Table 5). The isolated compounds showed a strong binding affinity when compared with inhibitors- pazopanib (-5.5 kcal.mol^{-1}), imatinib (-8.6 kcal.mol^{-1}) and batimastat (-10.1 kcal.mol^{-1}) of VEGF, TK, and MMP respectively. The amino acid residues in the binding pockets of protein VEGF are TYR21, GLN22, HIS27, TYR25, LEU97, GLU38, PHEA96, SER95, ASN75, HIS86, GLN89, GLY88, GLN87, PRO85, ILE46 and for protein TK -TYR149,GLY151, THR247, LYS104, PRO246, LYS245, LEU89, ASP365, ASN164, GLN362, ASN397, LEU398, ARG160, GLU157, GLU524, GLU159 and LEU163 and for protein MMP are ALA133, ASP129, ASN246, LYS13LYS241, LEU214, VAL235, THR239, PHE237, TYR240, LEU181, PRO238, GLU219, PHE237, ALA182, ZN264, HIS183, ILE180, THR215, PHE237, HIS222, THR205, ASP254, SER229, MET236, LEU226, ALA234, HIS228, ARG256 and GLY227. The above amino acids are responsible for various interactions between the above proteins and the ligands docked (Figs 3, 4 and 5). These amino acid residues are ligand-dependent and the hydrogen, vander waal, and various pi bonds stabilizing the interactions are present between the respective ligands and amino acid residue combinations present in the active site of the proteins. Several amino acid residues present in the binding pocket of proteins are involved in various interactions that occur between the ligand and their targets.

ADMET Analysis

In the preliminary stages of the drug discovery process, it is important to measure various indices of absorption, distribution, metabolism, excretion and toxicology (ADMET).[47] The physico-chemical properties of ligand molecules are detailed in (Table 6). The ADMET analysis is a key and considerable step, which eliminates lead compounds with the ability to elicit hazardous side-effects.[48] Laboratory experimentation of ADMET is very expensive and time consuming and hence *in-silico* ADMET evaluation is a much viable option that prevents potential drug failures during clinical trials.[49] The docked ligands such as azoprocarbazine, meptazinol and ecgonine illustrated in Fig. 3, showed positive results for human intestinal absorption (HIA), $CACO^{-2}$ permeability (CP), and blood-brain barrier (BBB) penetrability, while ecgonine was positive for HIA and CP and showed negative for BBB permeability. When a drug is administered orally, absorption of occurs primarily in the intestine. Transporter proteins, efflux proteins, and phase II conjugation enzymes are highly expressed by $CACO^{-2}$ cells in the intestine which make them useful models of transcellular pathways and metabolic biotransformation of test substrates.[44] The compounds that exhibit positive results for both HIA and CP indicated the absorption or assimilation of these compounds through human intestine. Compounds that exhibit positive BBB permeability might also be positive for pharmacological brain function, and none of the ligands were identified as P-gp substrates (Table 7). The safety of compounds is absolutely crucial for the development of a successful drug. Hence, drug like a candidate should satisfy most of the ADMET properties, which are as critical as therapeutic properties.[44,50] The toxicity prediction of meptazinol and ecgonine are found to be non-toxic (negative by Ames test. Table 8), except azoprocarbazine which showed a positive results. Azoprocarbazine, meptazinol and ecgonine are weak inhibitors of the human ether-a-go-go-related gene and hence negative for carcinogenicity effect on rat and mouse which suggested that the ligands are non-carcinogenic as well as non-mutagenic (Table 8).

Conclusion

The present work provides comprehensive evidence of the presence of compounds with multiple biological properties in the tuber extract of *J. heynei*. The above observations support the ethnomedicinal applications of the plant species for treating various diseases and disorders. The tuber extract of the plant showed significant anticancer activity *in-vitro* against DLA and EAC cell lines. In support of this, the docking studies showed a strong binding affinity of azoprocarbazine, meptazinol and ecgonine towards cancer-related proteins particularly kinase inhibitors and apoptosis-related proteins. Thus, the *in-silico* analysis revealed the potential of the screened compounds of *J. heynei* tuber to induce apoptosis and act as kinase inhibitors. Based on these studies, *in-vitro* experiments could be designed rationally to validate biological activities. The presence of several compounds with promising bioactivities upholds the importance of this plant species as a potential source of therapeutic agents.

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Ethical Approvals

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