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Original Research Article

Determination of allyl isothiocyanate from *Hedyotis herbacea* and its *in silico* screening for wound healing activity

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Abstract

Purpose: *To determine the bio-active constituents from the chloroform leaf extract of Hedyotis herbacea, and the wound healing activity of its phytoconstituents.*
Methods: *A gas chromatographic-mass spectrophotometer (GC-MS) was used to isolate and characterize the plant's chloroform extract. In order to verify the effectiveness of the isolated allyl isothiocyanate, it was docked with key enzymes needed for the healing process of wounds, such as p50 NF-κB, Interleukin-6 (IL-6), COX-2, TNF-α, Interleukin-1β, MMP-9, FGF-2, FGF2/FGF2-FGFR1, IGF1R, and TGF-β1 by using AUTODOCK VINA.*
Results: *The GC-MS isolated and identified 425 compounds in the screened extract, out of which 29 compounds were shown to have significant pharmacological activity. The allyl isothiocyanate compound present in the extract was shown to have the ability to repair skin damage. In silico analysis revealed that allyl isothiocyanate interacts efficiently with the active site of residues of amino acids found in the crucial proteins which are involved in wound healing process.*
Conclusion: *Hedyotis herbacea contains allyl isothiocyanate, which has wound-healing properties. Other unexplored phytocompounds in the extract might also contribute to the wound-healing activity of the plant. This should be further evaluated in order to access more wound-healing properties of the plant.*

Keywords: *Hedyotis herbacea, Phytoconstituents, GC-MS, In silico, Allyl isothiocyanate, Wound healing*

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INTRODUCTION

A glabrous, erect annual shrub, *Hedyotis herbacea Linn* is distributed throughout Asia and Africa's tropical and temperate zones. It belongs to the Rubiaceae family. Its leaves have been administered to abscesses, and wounds, and as an emollient [1-3]. *Hedyotis herbacea* has wound-healing properties in folklore medicine and has been used traditionally for decades. Exploring the pharmacological activities of the plant gives more authentication for the research work and the researchers. The plant possesses numerous phytoconstituents, which might be responsible for wound healing activity. Previous research works on *in vitro* and *in vivo* studies of wound healing activity have shown significant

effects of the chloroform extract of *Hedyotis herbacea* [4,5]. Thus, the present study was carried out to determine the GC-MS analysis of chloroform extract of *Hedyotis herbacea.* Isolate and identify the phytoconstituents which would be confirmed by *in silico* docking studies to promote wound healing activity.

Proteins involved in the inflammatory phase associated with injuries are NF-(κ)B, Interleukin-6 (IL-6), COX-2, TNF-α, and IL-1β. The genes involved in the initiation of the inflammatory response is transcripted by NF (κ)B (nuclear factor kappa-light-chain-enhancer of enabled B cells). Interleukin 6 (IL 6) is a multifunctional cytokine with major roles in inflammation, response to infections, metabolic regulation, and regenerative and neurological processes. COX-2 is generally not expressed in most cells but is significantly induced in response to an inflammatory stimulus, which tends to result in prostaglandin production, such as prostaglandin E-2 (PGE-2). The TNF-α disrupts fibroblast function, and an increase in TNF-α mast cells is linked to venous ulcers. IL-1β process via caspase-1 indicates the role of IL-1β in inflammation and pain throughout the duration of the condition. The MMP inhibitor had been an intriguing concept for the wound healing process. Proteins involved in the proliferation phase are Fibroblast growth factor (FGF), FGF-2 and FGFR-1, and IGF1R. Fibroblast Growth Factor (FGF) is a key player in a number of crucial biological processes, including cell division, migration, differentiation, morphogenesis, and angiogenesis. Fibroblast growth factor binds to tyrosine kinase receptor and to heparin sulfate (HS) receptor, a component of the proteoglycan sugar that diffuses into the cell and to the surface of the extracellular matrix.

Heparin sulphate protects FGF from inactivation and facilitates the binding of the FGF receptor, which then elicits biological signaling and interaction. In the FGF family, FGF-1 and FGF-2 are mainly sought after. Heparin which binds to FGF-2 and FGFR-1 acts as a stimulator of the growth factor, and heparin which binds only to FGF-2 acts as a signal inhibitor by taking up the growth factor. The insulin-like growth factor receptor tyrosine kinase (IGF1R), a member of the class of signaling molecules, is a crucial component of the signaling pathway involved in cell growth, proliferation, and survival. The protein involved in the remodeling phase is TGF-β, and it is involved in certain healing processes which include inflammation, stimulation of angiogenesis, proliferation of fibroblasts, collagen synthesis, and precipitation, in addition to a remodeling of the new extracellular matrix [6].

Based on the significance of the above-mentioned proteins in the wound healing process, the proteins were selected for autodock studies, in order to predict the wound healing property of phytoconstituents of the plant extract.

EXPERIMENTAL

Collection and authentication

Hedyotis herbacea Linn leaves were collected from the Tirunelveli district, Tamil Nadu, India and authenticated by V. Chelladurai, Research officer - Botany (Scientist-C), Centre Council for Research, Ayurveda and Siddha, Government of India. The voucher sample with specimen number: CS-BOT-HH-01 was kept in the herbarium.

Preparation of plant extract

The leaves were dried and pulverized to a fine powder. The crude powder was extracted using a range of polarity-increasing solvents, including petroleum ether, chloroform, ethyl acetate, and ethanol. The maceration approach was used to retain the 1.5 kg of crude powder in contact with the suitable solvents for 7 days at room temperature with occasional shaking. The extracts were then filtered, and the filtrates were concentrated under low pressure to provide the desired residue.

Gas chromatography-mass spectroscopic analysis

The GC-MS analysis of the chloroform extract of *Hedyotis herbacea* was performed using GC-MS (Agilent technologies GC:8890; MS:7000D GC/TQ). The equipment had an HP 5MS Ultra inert with dimensions of 30 mm x 0.25 mm ID x 0.25 µm film. The carrier gas used was helium (UHP grade) with a flow rate of 1.516 mL/min. The injector was operated at 280 °C, and the oven temperature was programmed as follows: 80 °C for 15 min, then gradually increased to 280 °C for 3 min. The identification of the components was based on NIST MS search V.2.3: 2017, likewise, their retention indices were compared. The constituents were identified after being compared to those in the computer library NIST and the findings were reported.

Molecular docking

AUTODOCK VINA was used to carry out the molecular docking study. Using Open Babel

2.4.0, small molecules file conversions were also made possible. Finally, Discovery Studio Visualizer version was used to complete the molecular visualization.

Target preparation

The RCSB PDB website was used to obtain the target's crystal structure (PDB ID: 1ALU; 1ITB; 1FQ9; 1SVC; 1VJY; 2AZ5; 2ZM3; 40EE; 5IKQ; 5UE4). The target molecule was then prepared for molecular docking using Auto Dock Tools target preparation scripts.

Ligand preparation

The ligands' SMILES file was translated to PDBQT format using Open Babel 2.4.0 with the creation of 3D coordinates option enabled.

Molecular docking

The ligands were initially docked globally using AutoDock Vina.

Visualization

The visualization was done by Biovia Discovery Studio client 2020.

RESULTS

The yields of the extracts were found to be Petroleum ether, 27.26 g; Chloroform extract, 32.85 g; Ethyl acetate, 18.23 g, and Ethanol, 8.72 g. A report from a previous study suggested that the chloroform extract possesses better activity than other extracts [4,5]. Therefore, the chloroform extract was used for further investigations.

Gas chromatographic-mass spectrometry (GC-MS) is one of the golden standard techniques for identifying and profiling secondary metabolites in both plant and non-plant species. Based on the polarity of solvents, *Hedyotis herbacea* was extracted, and all the extracts were examined using HPTLC to check for the presence of ursolic acid. The investigation of wound-healing activity used a chloroform extract high in ursolic acid with an emphasis on the phytoconstituent-based biological activity [7]. The research was carried out on chloroform extract in the *in vitro* assay (wound scratch assay) using H9c2 (2-1) cell line, and *in vivo* studies were also carried out on the excision and incision wound model [4,5]. Both *in vitro and in vivo studies* showed significant activity in the chloroform extract. Chloroform extract was more effective than the ursolic acid when isolated from the chloroform extract [4,5]. Hence the present GC-MS study was carried out to determine the bioactive compounds in the chloroform extract of *Hedyotis herbacea.* Apart from ursolic acid, which has wound healing property. GC-MS results showed that 425 phytoconstituents were present in the extract. Based on the literature survey, 29 compounds were identified to be pharmacologically active. The active principles were depicted in Figure 1, Figure 2 and Table 1.

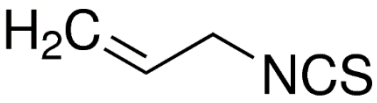

Figure 2: Structure of allyl isothiocyanate

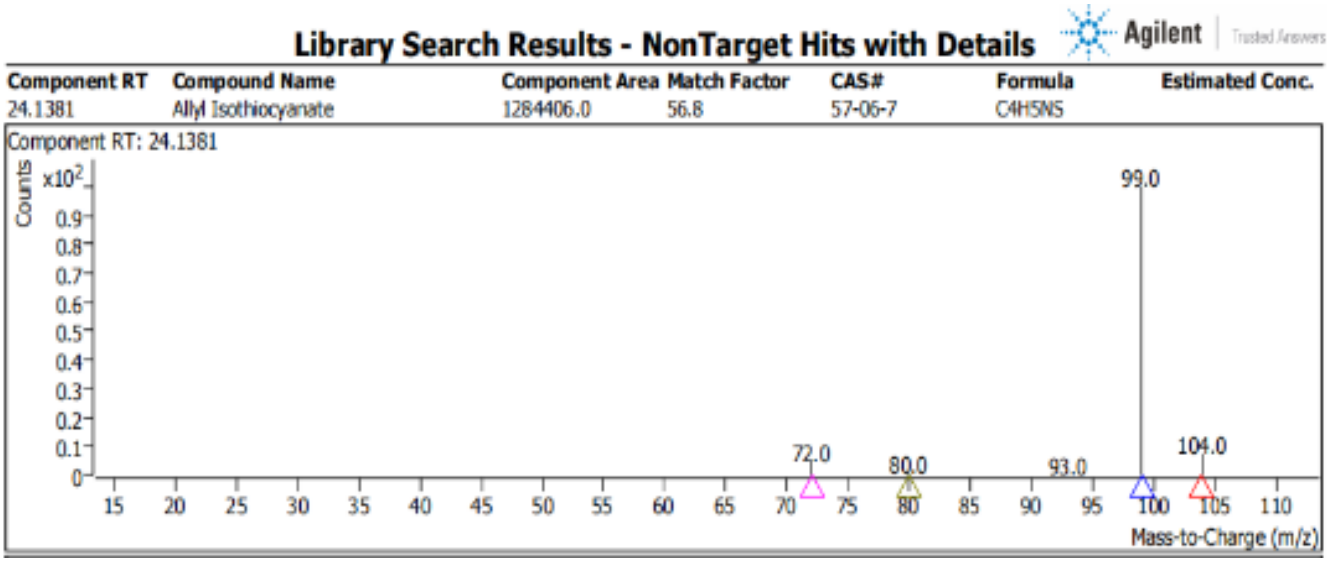

Figure 1: GC-MS data of allyl isothiocyanate peak

Table 1: Metabolite profile of chloroform extract of *hedyotis herbacea*

Retention time (unit)	Name of compound	Molecular formula	Peak area (unit)	Pharmacological activity	References
4.1863	Sarcosine, N-(2,6-difluorobenzoyl)-, octyl ester	$C_{18}H_{25}F_2NO_3$	1530415.4	Schizophrenia	[9]
25.6212	Neophytadiene	$C_{20}H_{38}$	318997486.7	Antimicrobial & Anti-inflammatory	[10]
31.0108	benzenesulfonamide, N-(4-hydroxyphenyl)-	$C_{12}H_{11}NO_3S$	3116075.9	Analgesic	[11]
25.6094	1H-Pyrrole-2,5-dione, 1-ethyl-	$C_6H_7NO_2$	1621693.5	Antioxidant	[12]
26.2060	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	40105836.8	Antidiabetic & Antioxidant	[13]
22.7361	4-Ethylbenzoic acid, tridec-2-ynyl ester	$C_{22}H_{32}O_2$	7644502.8	Anticancer	[14]
22.7988	Phenol, 3,4,5-trimethoxy	$C_9H_{12}O_4$	2243116.7	Anthelmintic	[15]
24.6408	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-8	$C_{11}H_{16}O_3$	39567638.5	Anti-inflammatory	[16]

The docking of ligand allyl isothiocyanate with 10 different proteins: (PDB ID:1ALU) p50 NF-κB, (PDB ID:1ITB) Interleukin-6 (IL-6), (PDB ID:1FQ9) COX-2, (PDB ID:1SVC) TNF-α, (PDB ID:1VJY) Interleukin-1β, (PDB ID:2AZ5) MMP-9, (PDB ID:2ZM3) FGF-2, (PDB ID:40EE) FGF2/FGF2-FGFR-1, (PDB ID:5IKQ) IGF-1R and (PDB ID:5UE4) TGF-β1 were done by AUTODOCK VINA software, and the dock scores of these molecules are represented in Table 2 and Figure 3, with their binding affinity and types of bonds, with which different amino acids were bonded to the ligand's different functional groups.

Table 2: The interaction energies (kcal/mol) of 10 proteins and ligand obtained from the molecular docking with autodock vina with PYRX

PBD ID	Name of the protein	Auto dock vina	H-Bonds	Van der Waals forces	Alkyl
		PyRx	Residues	Residues	Residues
1SVC	p50 NF-κB	-4.2	GLY P:64, GLY P:68, PRO P:65, ARG P:59, VAL P:115	LEU A:260, ARG A:255, MET A:253, THR A:251, PHE A:262, GLN A:250	ILE A:259
1ALU	Interleukin-6	-4.1	LEU A:167	SER A:37, HIS A:164, ARG A:168, ARG A:40	LYS A:171, ILE A:36
5IKQ	COX-2	-4.8	THR A:206	HIS A:386, LEU A:390, HIS A:207, GLN A:203, PHE A:210, ASN A:382	TRP A:387, TYR A:385, ALA A:202
2AZ5	TNF-α	-3.7	ASP D:143, TYR D:141	ASP D:140, GLN D:67, GLN D:66, PHE D:144, LEU D:142	TYR A:512
1ITB	Interleukin-1β	-4.1	TYR A:141, ASPA:143	GLN D:67, GLY D:66, PHE D:144, LEU D:142	LYS D:65
5UE4	MMP-9	-4.8	GLU A:47, GLN A:43	MET A:94, ARG A:51, GLY A:186, LEU A:187, LEU A:44	TYR A:52, TYR A:48
4OEE	FGF-2	-3.3	LYS A:26	GLN A:134, PRO A:132, ASN A:101, GLU A:99, LEU A:138, LYS A:135, GLY A:133	
1FQ9	FGF2/FGF2-FGFR1	-3.3		LYS C:172, LYS C:175, VAL C:174, HIS C:166, TYR C:243, LYS C:177	PHE C:176, VAL C:168
2ZM3	IGF1R	-4.2	GLU A:1080	GLY A:1152, LEU A:1005, ALA A:1031, ALA A:1031, LEU A:1081	MET A:1142, MET A:1079, VAL A:1063, MET A:1082
1VJY	TGF-β1	-4.0	LEU A:254, TYR A:249	VAL A:507, ASP A:509, ARG A:394, LYS A:519, GLN A:373, PHE A:374	LYS D:65

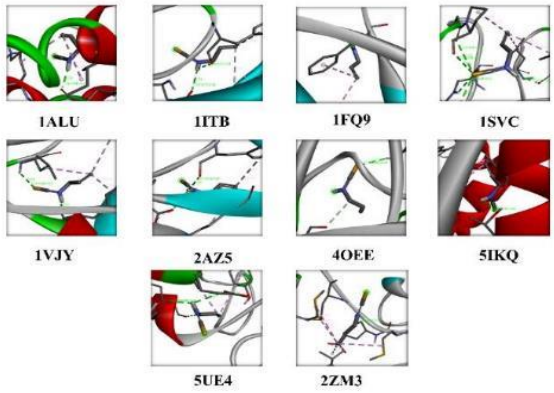

Figure 3: 3D Docking Poses of AUTODOCK VINA (Docking pose between allyl isothiocyanate and proteins)

DISCUSSION

The process of wound repair is intricate and unique, including several cells and cytokines. Injuries to the skin, which serves as a protective layer of the body, include scarring, infections, ulcers, and even chronic wounds [8]. Nowadays, *in silico* investigations are one of the approaches used to verify a compound's ability to heal wounds. The protein involved in the wound healing process was used to perform an *in silico* approach for the phytoconstituent allyl isothiocyanate found in the *hedyotis herbacea*.

In the studies, proteins were used as (PDB ID:1ALU) p50 NF-κB, (PDB ID:1ITB) Interleukin-6 (IL-6), (PDB ID:1FQ9) COX-2, (PDB ID:1SVC) TNF-α, (PDB ID:1VJY) Interleukin-1β, (PDB ID:2AZ5) MMP-9, (PDB ID:2ZM3) FGF-2, (PDB ID:40EE) FGF2/FGF2-FGFR1, (PDB ID:5IKQ) IGF1R and (PDB ID:5UE4) TGF-β. The aforementioned proteins were docked with allyl isothiocyanate using the AUTODOCK VINA software, and dock scores were used to demonstrate the efficacy of its wound healing ability.

The results of this study indicate that the wound healing activity of chloroform extract of *Hedyotis herbacea* leaves may be related to its high content of bioactive components. The GC-MS analysis of chloroform extract showed the presence of allyl isothiocyanate. The *in silico* analysis of allyl isothiocyanate, showed effective interaction with proteins which are involved in wound healing process. However other than allyl isothiocynante, compounds identified in the extract by GCMS are shown to possess antimicrobial activity. This property serves as a supporting factor for the wound healing potential of *Hedyotis herbacea.* The other unexplored phytoconstituents present in the extract would need further studies to examine their pharmacological potentials.

CONCLUSION

Hedyotis herbacea is a potential source of a wound healing agent due to the existence of pertinent phytochemicals and bioactive substances. It would be necessary to isolate the identified compound and evaluate its biological activities, as well as its mechanism of action.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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