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ORIGINAL ARTICLE

In silico analysis of *Typha domingensis* Pers. phytocompounds against wound healing biomarkers and ascertaining through in vitro cell migration assay

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Abstract

Typha domingensis Pers. is known for its medicinal properties. Although traditionally *T. domingensis* Pers. has been used for wound healing, yet scientific investigations reporting its ability to heal wounds are lacking. Phytochemical profiling of *T. domingensis* Pers. inflorescence crude extract was carried out by LC–MS analysis. Ten phytochemicals were selected for in silico analysis based on retention time, mass-to-charge ratio and resolution of mass spectrum. Molecular docking of all ten compounds was done against selected wound healing biomarkers viz., interleukin 6(IL-6), interleukin β (IL-β), insulin-like growth factor tyrosine kinase receptor (IGF-1R) and transformation growth factor β (TGF-β). Based on this, catechin, mesalazine and piperazine were subjected for in vitro cell migration assay (3T3 L1 mouse fibroblast cell line) to assess their wound healing potentials. Molecular docking revealed that mesalazine, catechin, and piperazine have potential ligands based on lowest docking energy (ranging from − 4.1587 to − 0.972), Glide E score (ranging from − 26.929 to − 57.882), Glide G score (ranging from − 4.16 to − 7.972) and numbers of hydrogen bonds compared to other compounds studied. The migration assay revealed that, compared to control (52.5%), *T. domingensis* Pers. inflorescence crude extract showed maximum wound healing potential (80%) followed by Catechin (66.8%) Mesalazine (58.3%) and Piperazine (51.2%). The combined in silico and in vitro approach opens new dimension for designing innovative therapeutics to manage different types of wounds.

Keywords *Typha domingensis* Pers · Molecular docking · LC–MS analysis · Cell migration · Scratch assay · Wound healing

Introduction

Nature has been a source of medicinal compounds for millennia. Medicinal plants have been used to treat various human diseases because of their therapeutic values (Nostro et al. 2000). There are approximately 2,50,000–5,00,000 plant species that exist on the earth (Borris 1996). Traditional medicines play a crucial role in primary health care in India. Approximately 80% of the world population relies on traditional medicines for their immediate health care needs. The reasons may be their efficacy, safety, cultural acceptability, and fewer side-effects. Moreover, the plant constituents have a better compatibility with the human body (Kamboj 2000). Medicinal plants comprise a wide variety of phytochemicals that are valuable because of their therapeutic properties that can be used for the treatment of human diseases. Using high-throughput screening methods and combinatorial chemistry, a great diversity of new drugs have been developed over the past 50 years (Ngo and Okogun 2013). Apart from treating various human diseases, medicinal plants are used for wound management.

Numerous drugs from the plant have been described in Ayurveda for their wound healing properties under the term Vranaropak. According to Ayurveda, wounds are discontinuation of skin membrane,which results in the formation of a scar for life after healing (Biswas and Mukherjee 2003). There are four sequential overlapping phases involved in the wound healing process: (1) hemostasis, which is initiated within several hours after an injury; (2) inflammation phase (duration 1–3 days); (3) proliferation phase (duration 4–21 days); and (4) remodeling phase (duration might vary from 21 days to 1 year) (Reinke and Sorg 2012). Deregulation of any of these steps results in impaired healing, leading

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to chronic hard-to-heal ulcers or excessive scarring, which presents a major and increasing health and economic burden to our society (Mustoe et al. 2006; Sen et al. 2009).

During the inflammatory stage, neutrophils and monocytes are mainly activated,which rapidly migrate at the site of skin injury. This phase overlaps mainly with hemostasis,which stops bleeding and reflects an early stage of wound healing (Eming et al. 2014). On reaching the site of injury, neutrophils produce cytokines such as tumor necrosis factor (TNF-α), interleukin1β (IL-1β), and interleukin 6 (IL-6) to amplify the inflammatory response (Eming et al. 2014). These cytokines exert debridement by releasing a variety of antimicrobial substances (Eming et al. 2014). After elimination of keratinocytes, another set of growth-factors such as fibroblast growth factor (FGF), kerationcyte growth factor (KGF), and insulin-like growth factor (IGF-1) stimulates proliferation of keratinocytes (Strbo et al. 2014). This inflammation subsides and marks the beginning of re-epithelization or the proliferation phase by restoring vascular network and forming granulation tissue.

In proliferation phase, migration and proliferation of keratinocytes is stimulated by nitric oxide synthesized by macrophages (Jacinto et al. 2001), growth factors and cytokines that includes EGF, KGF, and IGF-1 (Barrientos et al. 2008). The formation of granulation tissue occurs due to deposition of fibroblast cells. These cells migrate at wound site in response to various signals (cytokines and growth factors) (Schultz and Wysocki 2009; Hinz et al. 2007). In the final stage (remodeling phase) of wound healing, TGF- β plays a vital role in differentiation of fibroblast to myofibroblast, that aids inwound contraction (Hinz 2007).

Medication that helps in wound healing has been a topic of research since ages. Various herbal products have been used since long time for wound healing. There are reports stating that bandages and dressings soaked in natural honey cleanses the wound and also stimulates healing. There are reported Chinese herbal medicines that have been found to exert faster healing (Siavash et al. 2015 and Chen et al. 2010). Several fatty acids have been shown to aid in wound healing (Alexander and Supp 2014). However, the modeling and design of fact acting synthetic drugs for the healing of chronic wounds have grabbed much attention. Additionally, much effort has been given for the identification of new drug molecules that are effective in healing chronic wounds of different types.

The in silico methodology for design of new synthetic molecules that have improved therapeutic efficacy with a reduced toxicity profile have been addressed in current research. In silico technique ensures the prediction of the activity profile of untested molecules based on chemoinformatic tools (Schultz 2003). Among various chemoinformatic tool, docking concepts are widely employed for identifying ligand-based interaction of untested molecules in comparison to a docked receptor of known activity profile (Lengauer and Rarey 1996). The ligand–receptor based interactions obtained from docking studies provide a quantitative as well as qualitative insight for the identification of the essential molecular attributes of the ligand molecules for showing improved activity.

Molecular docking generates various possible adduct structures that are ranked and grouped together according to their energy score. Structure-based drug design provides structural models of the target proteins using homologous protein modeling softwares, NMR and X-ray diffraction. The physicochemical properties of drug binding sites on the receptor, such as electrostatic field, hydrophobic field, main residues and hydrogen bond sites can be analyzed with molecular modeling software (Lokesh et al. 2019).

Medicinal properties of *T. domingensis* Pers. have been reported in many traditional literatures in India, China and Turkey. It has been used in the treatment of hematemesis, hematuria, uterine bleeding, postpartum abdominal pain, gastralgia, dysmenorrhea, and so on (Yeng-Himche 1985). It is also used as an anti-inflammatory agent (Kolhe et al. 2011). It has been reported that naringenin, an active component present in *T. domingensis* Pers. inhibits vascular smooth muscle cell proliferation; thus, it is used as a therapeutic agent in controlling vascular problems (Jung-Jin et al. 2012).

Despite of traditional worldwide recognition of *Typha* species in the wound management, a reference survey revealed that an in silico and i*n vitro* wound healing study had not been conducted so far. The study aimed to investigate the phytochemicals present in the crude extract of *Typha domingensis* Pers. inflorescence using liquid chromatography–mass spectrometry (LC–MS) for metabolite profiling, molecular docking and in vitro cell migration studies to ascertain the potent of the compounds to manage wound and related ailments.

Materials and methods

Collection of plant material

Aerial parts (inflorescences) of *Typha domingensis* Pers. were collected from the campus of Uka Tarsadia University, Bardoli, India. The plant was duly identified and authenticated (voucher no. CGBIBT/SAHA/ 019) in the herbarium collection of Department of Botany, Botanical survey of India, Jodhpur, Rajasthan, India. The collected inflorescences were dried in shadow at around 25–30 °C. The dried inflorescences were stored at 4 °C for further process.

Preparation of plant extract

Aqueous extract was prepared from 100 g shade dried *Typha domingensis* Pers. inflorescences using Soxhlet. After extraction, the extract was filtered using Whattman No.1 filter paper. The filtrate was concentrated using rotavapor at 40–60 °C. The crude extract was preserved at 4 °C for further analysis.

Phytochemicals profiling using LC–MS

The aqueous crude extract was subjected to LC–MS analysis using Q Exactive Plus Biopharma, ThermoScientific at Sophisticated Advanced Instruments Facility (SAIF), IIT Bombay. A data acquisition and interpretation software–ThermoScientific Xcalibur™ (version 4.2.28.14) was used for analysis. The column used for analysis consisted of Hypersil Gold 3 Micron 100* 2.1 mm. In the case of solvent composition, mobile phase A consisted of 0.1% formic acid in Milli-Q water, whereas solvent B consisted of methanol. Total run time used for elution of compounds was 30 min. Information Dependent Acquisition-Mass Spectrum (IDA-MS) analysis was performed in negative and positive ionization mode for all phytocompounds (Ali et al. 2016).

In silico screening of wound healing potential of selected phytochemicals obtained through LC–MS analysis

Preparation of ligands

Two-dimensional structures of the 10 selected phytochemicals from the LC–MS analysis of *Typha domingensis* Pers. inflorescence aqueous extract were obtained in SDF format from PubChem database (https://pubchem.ncbi.nlm.nih.gov/). They were further subjected to LigPrep module of Schrödinger Maestro v10.1, and later converted into a 3D structure by including ionization, variation, stereochemical, correction, optimization of geometry, and energy minimization. Minimization was carried out using optimized potentials for liquid simulation—2005 force field (OPLS_2005). The optimized ligands were further used for molecular docking (Kalirajan et al. 2012).

Preparation of receptors

Selection of receptors for this study was made on the basis of their involvement in the process of wound healing. The X-ray crystallographic structures of all selected receptors involved in the process of wound healing were downloaded from the Protein Data Bank (https://www.rcsb.org). Receptor preparation was carried out using the protein preparation wizard of Maestro provided by Schrödinger. Further, the protein structures were refined by assigning their bond orders, adding their missing hydrogen atoms and disulfide bonds, and removing water molecules (Kalirajan et al. 2012).

Generation of receptor grid

The ligand–receptor interaction was studied by receptor grid generation. The dimension of grid box was centered to enclose the site of ligand interaction and active sites of protein receptor.The native ligands in the receptor were excluded from grid generation, and the site of docked ligand was confined to the enclosing box (Kalirajan et al. 2012).

Molecular and glide docking analysis

For docking analysis, Schrödinger AutoDock software was used. After assigning Gasteiger charges, ligand files were saved into the PDBQT file format. Docking was carried out with 20 poses to interpret highly potential ligand residue. Also, final scoring was carried out using the lowest Glide E score and lowest Glide G score, and the number of H-bonds for each ligands were recorded. Docked structures were analyzed in the Maestro visualization tool (Halperin et al. 2002).

HPLC quantification of phytocompounds

The concentration of *Typha domingensis* Pers. inflorescence crude extract was selected based on cytotoxicity test carried out through MTT assay using 3T3-L1 mouse fibroblast cell line and the concentrations of other test drugs like Catechin, Mesalazine and Piperazine were selected based on HPLC quantitation that consisted of Perkin Elmer Series 200 Diode array detector, UV detector and fluorescence detector. Chromatographic separation was achieved using mobile phase consisted of acetonitrile and phosphoric acid (pH 2.0) in the ratio of 90:10 for catechin, for mesalazine mobile phase consisted of phosphate buffer and methanol in the ratio of 60:40, and for piperazine mobile phase consisted of acetonitrile, diethylamine and methanol in the ratio of 90:5:5. The flow rate of 0.8 ml/min was used for all the compounds. The column was maintained at 30ºC temperature, and injection volume used was 20 µl.

In vitro scratch assay using fibroblast cell line

For in vitro scratch assay, 3T3- L1 mouse fibroblast cell lines were plated in 12-well plate to form a monolayer and incubated at 37 ºC, 5% CO_2 for 24 h.Once the cells reached around 70% confluence, a scratch was made to form wound.The monolayer was washed with 1 ml DPBS, and 2 ml growth medium was added to each well. Additionally, test compounds like aqueous crude extract of

Typha domingensis Pers. inflorescence (800 µg/ml), Catechin (35 µg/ml), Mesalazine (60 µg/ml) and Piperazine (130 µg/ml) were added and control (without additional drugs) to evaluate the effects on 3T3 L1 mouse fibroblast cell line, respectively. The cell cultures were incubated at 30ºC for 24 h. The images were captured under an inverted phase-contrast microscope equipped with a camera (Nikon, Japan) at regular time intervals of 0 h, 12 h and 24 h. Percentage of cell migration from control and treated cells were calculated and final gap (24 h) width to initial gap width (0 h) were compared (Cory 2011).

Results

LCMS analysis of aqueous crude extract of *Typha domingensis* Pers. inflorescence

The profiling of aqueous crude extract of *Typha domingensis* Pers. inflorescences by LC–MS, revealed the presence of phytochemicals belonging to groups such as alkaloid, tannins, phenols, and sequiterpene. Total 10 phytochemicals were manually selected based on retention time (Fig. 1), literature survey, and SimBioSys Lasso database (Table 1). These were used as ligands for molecular docking study.

In silico analysis of selected compounds

Three-dimensional structures of the selected ligands were used for analysis (Fig. 2). The interactions of these selected compounds were carried out against the selected biomarkers involved in different phases of wound healing such as IL-6, IL-1β, IGF-1R, and transformation growth factor β (Table 2). These selected biomarkers are mainly involved in (1) blood clotting and inflammation, (2) formation of new tissue or proliferation phase, and (3) tissue remodeling phase (Guo and Dipietro 2010; Werner and Antsiferova 2016).

Comparative docking analysis was based on docking energy, hydrogen bond interactions, and glide score of the selected 10 (phytocompound) ligands suggest that three compounds (mesalazine, catechin and piperazine) have potential ligands (Table 3). These compounds interact best with receptor IL-6 (PDB ID: 1ALU) involved in the inflammation phase of wound healing with Glide scores – 38.5, – 39.056 and – 36.705 kcal/mol respectively. Amino acid residues involved in the interaction were mainly MET 184, GLU 80, GLU 81, LEU 84, LEU 133, ASN 132, LYS 131, ALA-130 and LYS 129 and ALA 130 (Table 3; Fig. 3a–c).

Glide score value of interacting mesalazine, catechin and piperazine with receptor IL-1β (PDB ID: 1ITB) involved in inflammation phase of wound healing were found to be – 26.929, – 40.486 and – 32.405 kcal/mol, respectively.

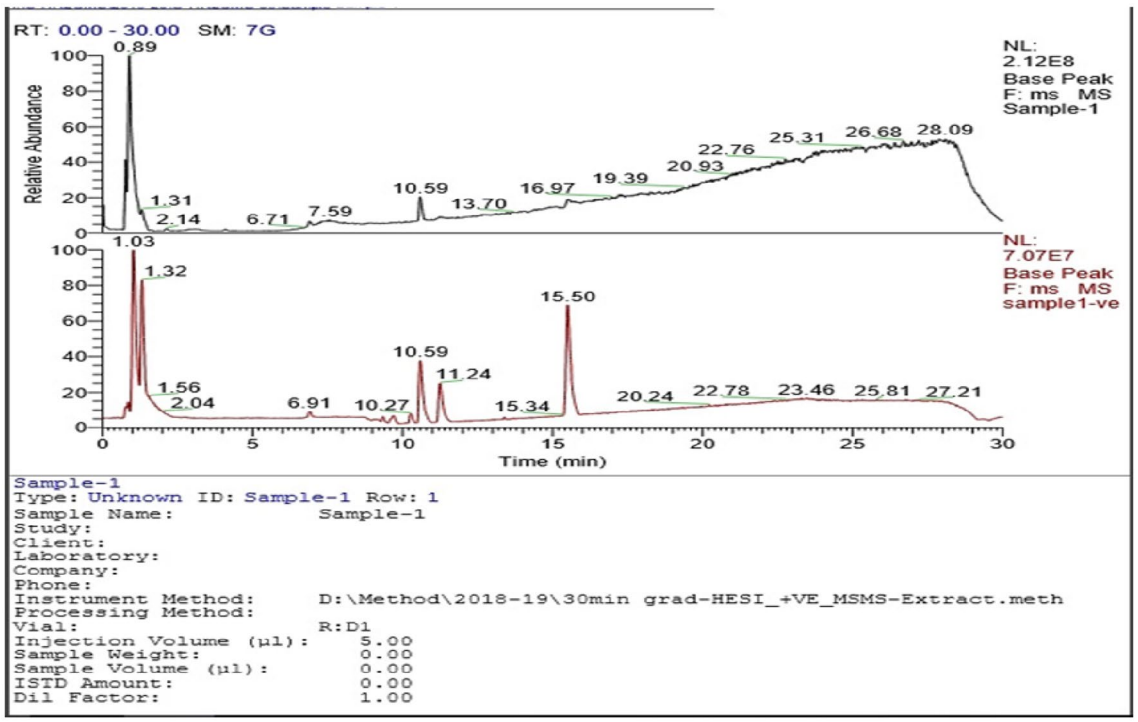

Fig. 1 LC–MS chromatogram of the aqueous extract prepared from inflorescences of *Typha domingensis* Pers

Table 1 Selected phytochemicals present in *Typha domingensis* Pers. inflorescence aqueous crude extract obtained by LC–MS analysis for in silico analysis

Sr. No	Phytochemicals	Molecular formula	Molecular Weight	Retention Time	PubChem/ Chem-spiderID
1	1,2-Dihydro-5-acenaphthylenyl(2,3-dihydro-1H-indol-1-yl) methanone	C21 H17 N O	299.1298	1.31	602,371
2	Indoline	C8 H9 N	119.0734	2.134	10,328
3	Benzenesulfonamide, N-[1-(1-adamantyl)propyl]-4-methoxy-	C20 H29 N O3 S	363.1885	6.764	585,391
4	Mesalazine	C7 H7 N O3	153.0424	7.603	4075
5	2-Hydroxycinnamic acid	C9 H8 O3	146.0366	10.59	637,540
6	*N*,*N*'-Diisopropyl[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine	C10 H16 N6 O	236.1386	13.737	535,977
7	Polygodial	C15 H22 O2	234.1616	19.347	72,503
8	Berberine	C20 H18 N O4	336.1225	22.776	2353
9	Catechin	C15 H14 O6	336.0846	6.904	9064
10	Piperazine	C17 H15 Cl F2 N2O	336.0846	10.272	4837

The ligands mainly involved were THR 51, GLU 52, GLN 53, GLN 59, HIS 60, LYS 61, GLU 62 and GLN 59 (Table 3; Fig. 4a–c).

The interaction with receptor TGF-β (PDB ID-1VJY) principally involved in the remodeling phase of wound healing process showed Glide score values of − 35.549, − 33.818 and − 45.262 kcal/mol, respectively. Amino acid residues mainly involved in interaction were ASP 281, TYR 282, HID 283, ALA 230, ALA 350 and ASP 351 (Table 3; Fig. 5a–c).

Interaction of compounds with IGFR1 (PDB ID: 2ZM3) involved in the proliferation phase of wound healing process showed Glide score values of − 38.896, − 57.882, and − 54.48 kcal/mol, respectively. The surrounding amino acid residues found to be ASP 45, ARG 44, TRP 28, GLN 27, TYR 151, TYR 119, SER 60, and GLY 121 (Table 3; Fig. 6c).

The interaction of selected ligands (mesalazine, catechin and piperazine) with all selected biomarkers involved in wound healing were found to be very strong. The strength was mainly due to formation of more number of hydrogen bonds with surrounding aminoacids (Table 4).

HPLC Quantification of crude extract

Based on in silico analysis, three phytocompounds (mesalazine, catechin and piperazine) were subjected to HPLC analysis. To determine the quantity of chosen phytocompounds present in aqueous crude extract of *Typha domingensis* Pers. inflorescence. The results of HPLC study (Table 5) revealed that the quantity of catechin was 42.22 mg/1 g of crude extract; mesalazine was 76.18 mg/1 g of crude extract and that of piperazine was 160.44 mg/1 g of crude extract. Quantification of compounds were based on peak area and retention time obtained after HPLC analysis.

Cell migration assay using selected phytochemicals

The cell migration assay was performed using 3T3 L1-mouse fibroblast cell line. The aqueous crude extract from *Typha domingensis* Pers. inflorescence and selected compounds (catechin, mesalazine and piperazine) were subjected for in vitro scratch assay. The results (Fig. 7) revealed that cells treated with aqueous crude extract of *Typha domingensis* Pers. inflorescence showed a highest wound healing (80.0%) as compared to the control (52.5%). The compounds catechin and mesalazine showed moderate wound healing activity (66.8% and 58.3%, respectively), followed by piperazine (51.2%).

Discussion

Several reports have stated that phytochemicals present in the medicinal plants are responsible for wound healing (Stephen et al. 2010; Pirbalouti et al. 2010; Subhashini and Arunachalam 2011; Dewangan et al. 2012). Komakech et al. (2019) reported the wound healing potential of *Aspilia africana.* These authors reported the presence of phytochemicals including alkaloids, saponins, tannins, flavonoids,terpenoids, β-caryophyllene, germacrene D, α-pinene, careen, phytol, and linolenic acid and linked them with anti-inflammatory, antimicrobial, and antioxidant activities, which are essential for wound healing. Nascimento (2018) also reported, the phytochemical extracts from a popular Brazilian folk medicinal plant *Ouratea fieldingiana* have wound healing potentials.

Taxonomic studies of *Typha* sp. are mainly limited to specific countries, viz., India (Saha 1968), Europe (Cook 1980), Iran (Bokhari 1983), Pakistan (Hamdi and Assadi 2003),

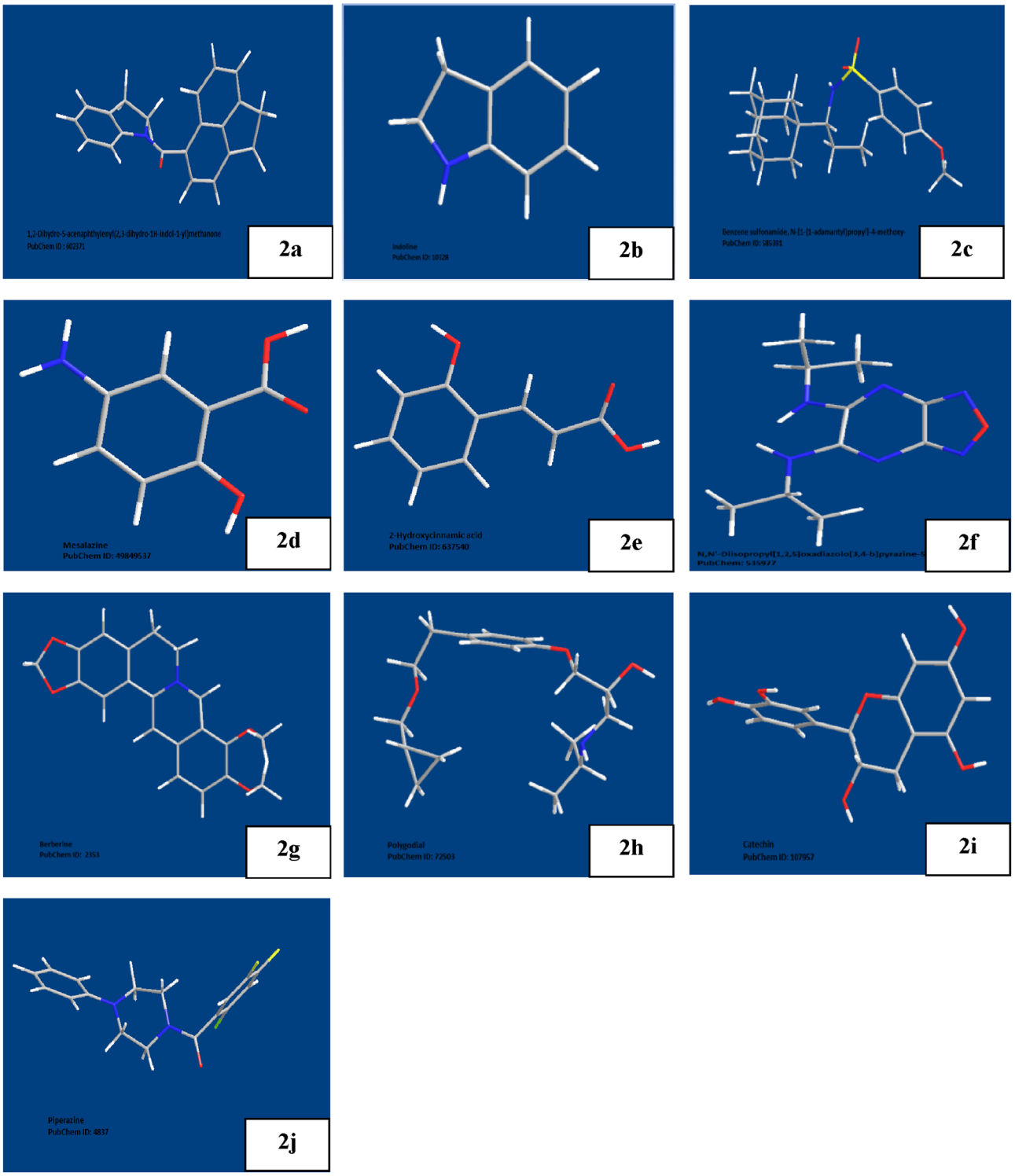

Fig. 2 Three-dimensional structures of identified phytochemicals downloaded from pubchem chemical database **a**. 1,2-Dihydro-5-acenaphthylenyl(2,3-dihydro-1H-indol-1 yl)methanone; **b** Indoline; **c**. Benzenesulfonamide, *N*-[1-(1-adamantyl)propyl]-4-methoxy; **d**. Mesalazine; **e**. 2-Hydroxycinnamic acid; **f**. *N*,*N*'-Diisopropyl[1,2,5] oxadiazolo[3,4-b]pyrazine-5,6-diamine; **g**. Polygodial; **h**. Berberine; 2i.Catechin; 2j.Piperazine

Table 2 Targets/receptors involved in wound healing phases

Sr. No	Name of Receptors	Wound healing Phases	PDB ID
1	Interleukin 6(Il-6)	Inflammation phase	1ALU
2	Interleukin β (Il- β)	Inflammation phase	1ITB
3	Insulin like growth factor tyrosine kinase receptor (IGF1R)	Proliferation phase	2ZM3
4	Transformation growth factor β (TGF- β)	Remodeling phase	1VJY

Table 3 Docking energy, gliding energy and hydrogen bonding interaction between target protein and phytocompounds

Ligands		Receptors PDB ID			
		1ALU`	1ITB	1VJY	2ZM3
1,2-Dihydro-5-acenaphthylenyl(2,3-dihydro-1H-indol-1-yl)methanone	Docking energy	− 3.644	− 3.283	− 5.888	− 5.483
	Glide G score	− 3.756	− 3.396	− 5.301	− 6.001
	Glide E score	− 31.153	− 22.281	− 14.718	− 28.647
Indoline	Docking energy	− 4.712	− 3.401	− 5.913	− 5.301
	Glide G score	− 4.712	− 3.401	− 5.301	− 5.913
	Glide E score	− 25.146	− 12.017	− 24.766	− 30.759
Benzenesulfonamide, *N*-[1-(1-adamantyl)propyl]-4-methoxy-	Docking energy	–	–	–	–
	Glide G score	–	–	–	–
	Glide E score	–	–	–	–
Mesalazine	Docking energy	− 5.46	− 4.357	− 6.285	− 6.098
	Glide G score	− 5.461	− 4.358	− 6.099	− 6.285
	Glide E score	− 38.4	− 26.929	− 35.549	− 38.896
2-Hydroxycinnamic acid	Docking energy	− 4.43	− 4.333	− 4.914	− 5.463
	Glide G score	− 4.43	− 4.333	− 5.463	− 4.914
	Glide E score	− 29.134	− 30.833	− 32.896	− 29.197
N,N'Diisopropyl[1,2,5]oxadiazolo[3,4-b]pyrazine-5,6-diamine	Docking energy	–	–	–	–
	Glide G score	–	–	–	–
	Glide E score	–	–	–	–
Polygodial	Docking energy	− 3.298	− 3.2020	–	− 5.468
	Glide G score	− 3.298	− 3.202	–	− 5.468
	Glide E score	− 16.275	− 16.161	–	− 25.741
Berberine	Docking energy	− 3.116	− 3.516	− 5.505	− 5.429
	Glide G score	− 3.116	− 3.516	− 5.429	− 5.505
	Glide E score	− 27.18	− 22.196	− 21.365	− 41.729
Catechin	Docking energy	− 5.035	− 5.072	− 7.972	− 6.86
	Glide G score	− 5.035	− 5.072	− 6.86	− 7.972
	Glide E score	− 39.056	− 40.486	− 33.818	− 57.882
Piperazine	Docking energy	− 4.244	− 4.158	− 5.951	− 6.245
	Glide G score	− 4.244	− 4.16	− 6.247	− 5.952
	Glide E score	− 36.705	− 32.405	− 45.262	− 54.48

Australia (Finlayson et al. 1985), China (Sun and Simpson 2010), and North America (Kuehn and White 1999). Since 1978, approximately 15 species have been reported (Govaerts 2018). It has been reported that *Typha domingensis* Pers. pollens are traditionally used to prevent bleeding (Bensky et al. 1993). Thus, knowing this fact, the present study was initiated to know which compounds present in *Typha domingensis* Pers. inflorescences were involved in wound healing. Treatments that are used for healing wounds mainly focus on controllable factors through clearance of infection, mechanical protection, and through nutritional support. Thus, the identification of therapeutic targets and

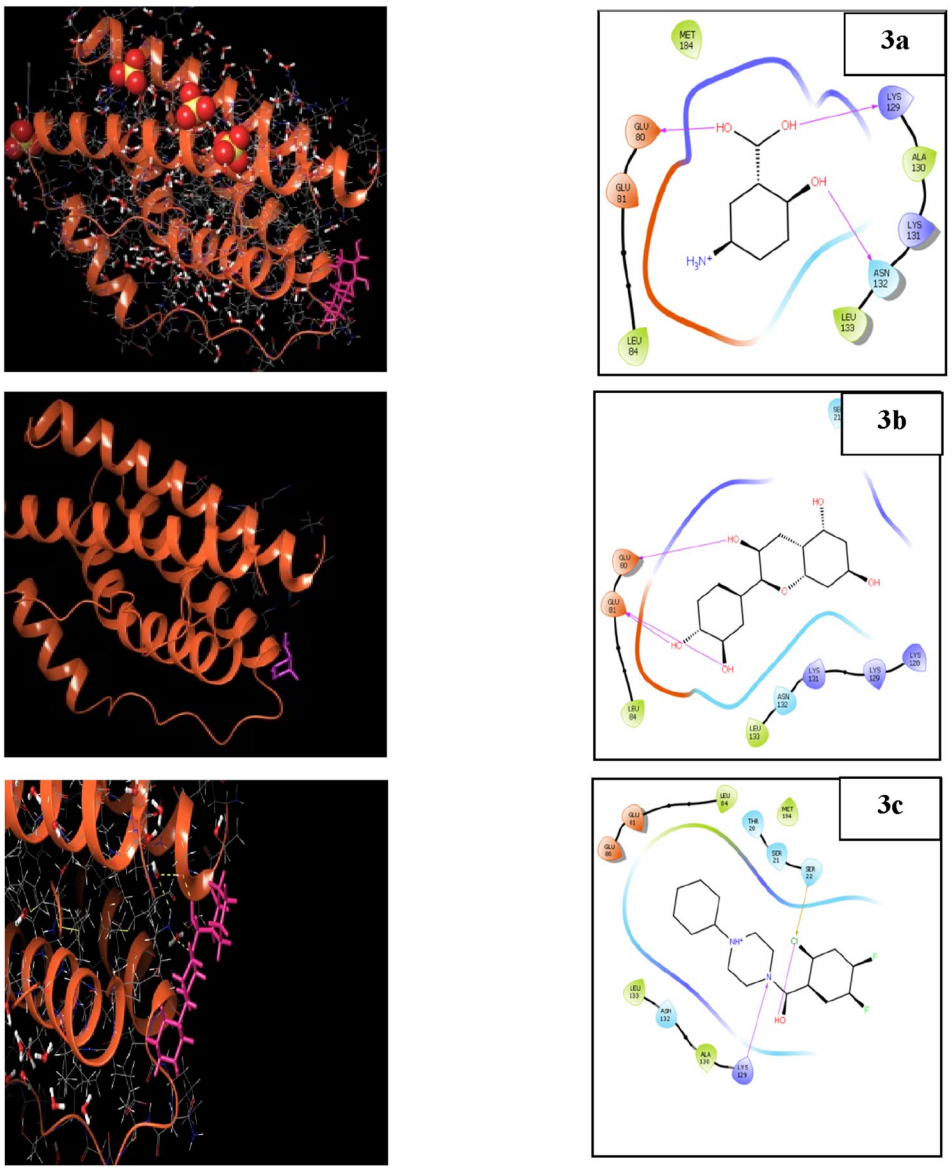

Fig. 3 Molecular interaction with Interleukin 6 (PDB ID 1ALU) involved in inflammation phase of wound healing process. **a** Mesalazine; **b** Catechin; **c** Piperazine

the development of more effective treatments are required (Sun et al. 2014).

Molecular docking is an attractive scaffold to understand drug biomolecular interactions for the rational drug design and discovery. The mechanistic study by placing a molecule (ligand) into the preffered binding site of the target specific region of the protein (receptor). The ligand–receptor form a stable complex of potential efficacy and more specificity (Rohs et al. 2005; Guedes et al. 2014). Molecular docking requires data bank for the search of target with proper PDB format and a methodology to prepare lugand as a PDB file. Docking of small molecules to a target includes a predefined sampling of possible conformation of ligand in the particular groove of target in an order to establish the optimized conformation of the complex. This can be made possible using scoring function of software (Seeliger and de groot 2010).

Variety of interactions that influence wound healing requires the need for computational models to understand and investigate this complex process of wound healing (Ziraldo et al. 2013). Numerous work have been conducted to design such models for various stages of wound healing processes (Ziraldo et al. 2013). These models can then be validated experimentally and used to assist in various areas of tissue engineering research (Ziraldo et al. 2013).

The computational strategies have gained an intense value in pharmaceutical research, due to their ability

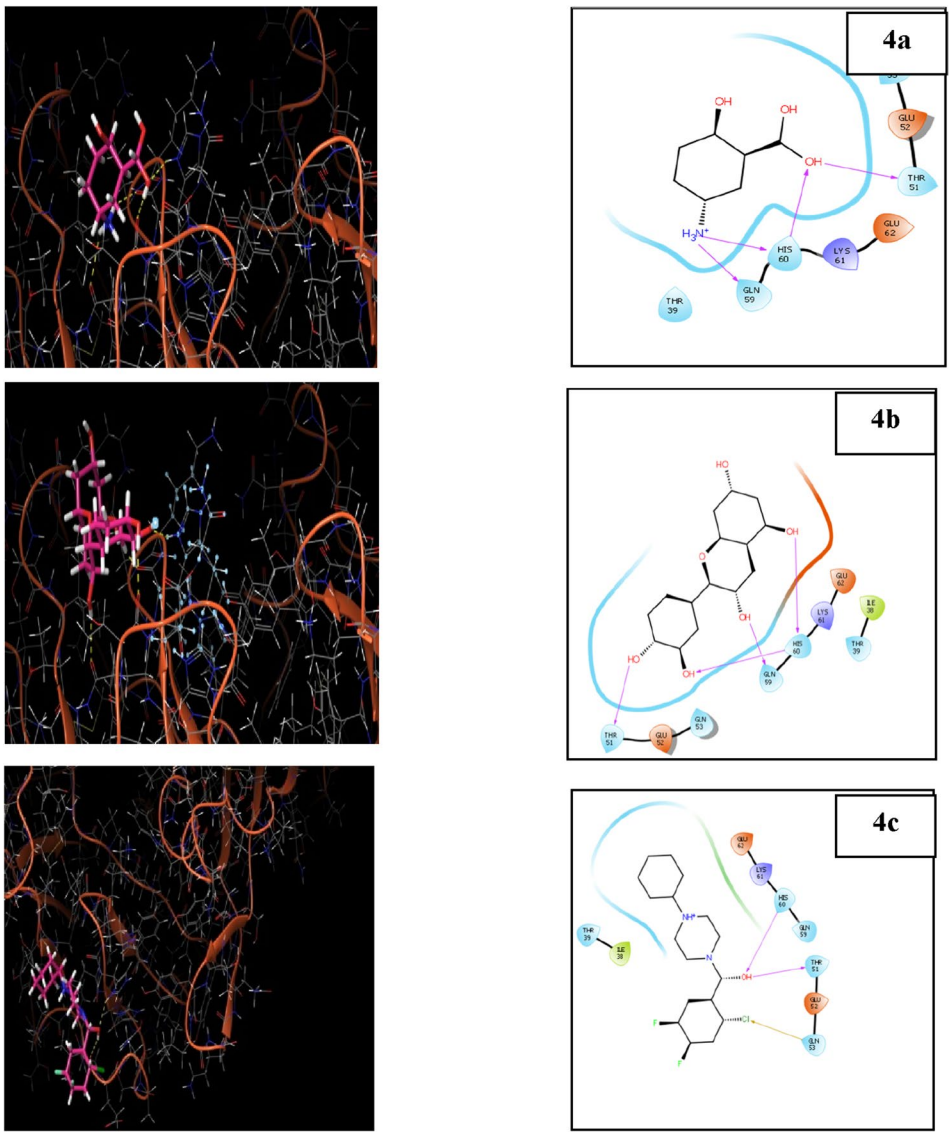

Fig. 4 Molecular interaction of with Interleukin β (PDB ID 1ITB) involved in inflammation phase of wound healing process. **a** Mesalazine; **b** Catechin; **c** Piperazine

to identify and develop novel promising compounds by molecular technique (Lounnas et al. 2013; Yuriev and Ramsland 2013). Many scientists from research groups have applied these techniques to identify potential novel compounds against varety of diseases (Ferreira et al. 2015).

The molecular docking was carried out to determine interaction of selected phytocompounds obtained through LC–MS analysis against selected wound healing biomarkers.

The molecular docking study revealed that, mesalazine interact significantly with all the selected markers studied in this work. It has been reported (Moss and Peppercorn 2007; Gisbert et al. 2002) that mesalazine, also known as 5-aminosalicylic acid (5-ASA) or mesalamine, is an anti-inflammatory drug used for the treatment of inflammatory bowel disease, inflamed anus, or rectum. Oh-Oka et al. (2017) reported that 5-ASA or mesalazine has anti-inflammatory property mainly because it helps to activate TGF-β1. An earlier report (Desmoulière et al. 1993) also states that activation of TGF-β1 helps in fibroblast contraction. TGF-β1 aids in determining the angiogenic properties of endothelial progenitor cells that supply blood and induce keratinocyte migration to the injured site (Gailit et al. 1994; Evrard et al. 2012).

On the basis of molecular docking analysis, catechin can be considered as the second best compound for the wound healing process. This compound also interacted with the selected biomarkers of wound healing phases in the best possible manner. The results were evaluated and confirmed based on docking energy, gliding energy, and the number of hydrogen bonds. Based on literature survey, it was found that catechins are naturally occurring polyphenolic compound

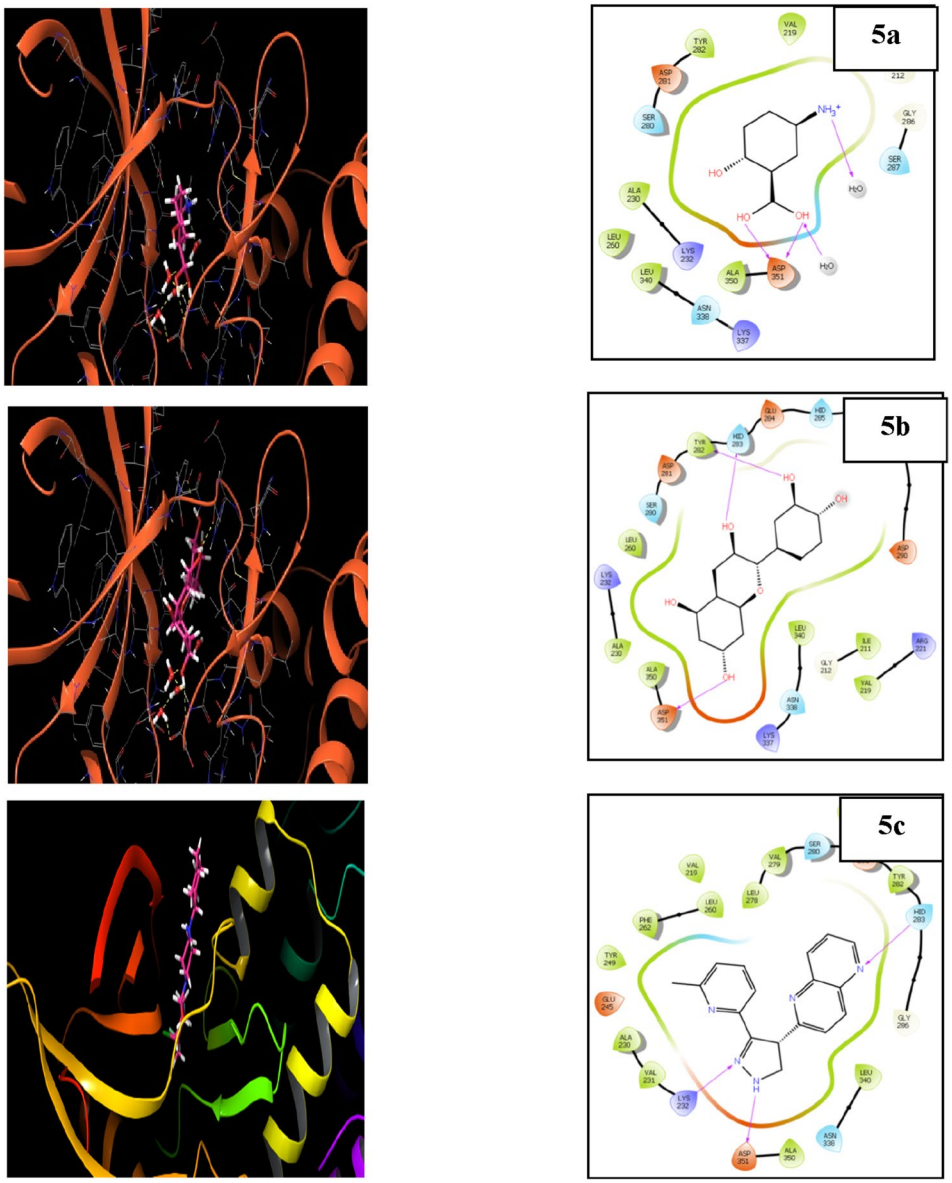

Fig. 5 Molecular interaction with TGF β (PDB ID 1VJY) involved in remodeling phase of wound healing process. **a** Mesalazine; **b** Catechin; **c** Piperazine

known for their anti-inflammatory, antioxidant, and free radical scavenging potentials (Yen and Chen 1998; Chan et al. 1999). Previous reports (Yang et al. 1998; Crouvezier et al. 2000) supports the finding of present study that, catechins increases the production of pro-inflammatory cytokines namely IL-1 and TNF. These pro-inflammatory cytokines inhibits the production of matrix metalloproteinase MMP-9, that causes delay in wound healing.

In the case of piperazine, the efficacy might be dependent on other factors that are required for cell migration. Moreover, the process of cell migration is a complex process that occurs due to the involvement of hundreds of molecules. The factors affecting the promotion of directional migration include cellular polarity machinery, receptor signaling, integrin trafficking, actomyosin contraction, etc. (Horwitz and Webb 2003; Petrie et al. 2009). It has been reported that piperazine and its analogs are important pharmacophores used in different therapeutics (Berkheij et al. 2005). Earlier researchs (Alireza et al. 2006; Chandra et al. 2006) have shown that piperazine analogs are known to possess antibacterial activity. The antibacterial properties of piperazine could be the reason behind its involvement in the wound healing process alternatively decreasing the microbial loads on the wounds and thus aiding in faster healing.

Based on in silico analysis, three phyto-chemicals (mesalazine, catechin and piperazine) were subjected for HPLC analysis to determine the quantity of

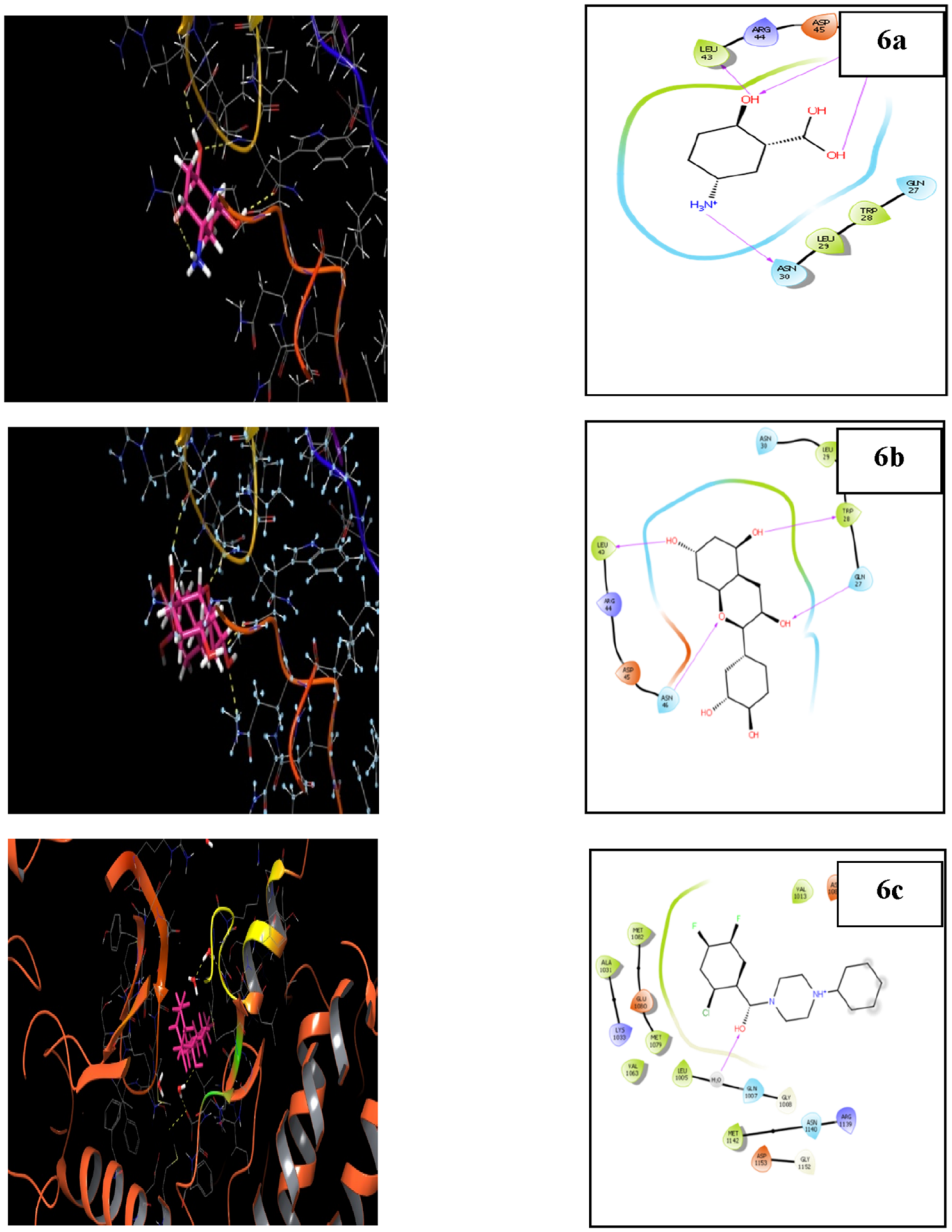

Fig. 6 Molecular interaction with IGFR1 (PDB ID 2ZM3) involved in proliferation phase of wound healing process. **a** Mesalazine; **b** Catechin; **c** Piperazine

chosen phytochemicals present in crude aqueous extract of *Typha domingensis* Pers. inflorescence. The results of HPLC study revealed that the quantity of catechin was 42.22 mg/1 g of crude extract; mesalazine was 76.18 mg/1 g of crude extract and that of piperazine was 160.44 mg/1 g of crude extract. Quantification of compounds was based on peak area and retention time obtained after HPLC analysis.

The wound healing assay (scratch assay) is the method of choice for studying cell migration due to its low cost and simplicity of its experimental design (Kramer et al. 2013; Liang et al. 2007). Monolayer of cells are grown to confluence in a multiwell assay plate. After creation of wound in the monloyer, migration of cells are assessed by monitoring the recolonization of the scratched region to quantify cell migration (Liang et al. 2007). This technique is maily used to understand the molecular mechanisms that affect cell migration (Simpson et al. 2008; Walter et al. 2010) and to identify pharmaceutical compounds that can modulate cell migration and consequently drive treatment therapies (Decaestecker et al. 2007).

The aqueous crude extract from *Typha domingensis* Pers. inflorescence and selected compounds (catechin, mesalazine and piperazine) were subjected for in vitro

Table 4 Docking energy, gliding energy and hydrogen bonding interaction between target protein and phytocompounds present crude aqueous extract

Sr. No	Ligand	Receptor (PDB ID)	Docking energy	Glide E Score	Glide G Score	No. of H-bonds
1	Mesalazine	1ALU	− 5.46	− 38.4	− 5.461	3
		1ITB	− 4.357	− 26.929	− 4.358	4
		1VJY	− 6.098	− 35.549	− 6.099	4
		2ZM3	− 6.285	− 38.896	− 6.285	4
2	Catechin	1ALU	− 5.035	− 39.056	− 5.035	3
		1ITB	− 5.072	− 40.486	− 5.072	4
		1VJY	− 6.86	− 33.818	− 6.86	3
		2ZM3	− 7.972	− 57.882	− 7.972	4
3	Piperazine	1ALU	− 4.244	− 36.705	− 4.244	2
		1ITB	− 4.158	− 32.405	− 4.16	2
		1VJY	− 5.951	− 45.262	− 6.247	3
		2ZM3	− 6.245	− 54.48	− 5952	1

Table 5 HPLC quantification of selected compounds based on peak area and retention time

Compound Name	Peak Area (uv* sec)	Retention time (Min.)	Quantity in mg/ 1 gm crude extract
Standard Catechin	2,387,362.85	1.806	–
Catechin in crude extract	100,800.09	1.784	42.22
Standard Mesalazine	9,512,819.18	1.784	–
Mesalazine in crude extract	724,870.61	1.873	76.18
Standard Piperazine	1,181,434.90	2.259	–
Piperazine in crude extract	189,566.63	2.010	160.44

scratch assay. The results revealed that cells treated with aqueous crude extract of *Typha domingensis* Pers. inflorescence showed a highest wound healing (80.0%) as compared to the control (52.5%). The compounds catechin and mesalazine showed moderate wound healing activity (66.8% and 8.3%, respectively), while piperazine (51.2%) failed to show any better wound healing as compared to control.

In conclusion, the present in silico study investigated the efficacy of selected 10 phytochemicals obtained through LC–MS analysis to heal wounds. Molecular docking of all ten compounds was done against selected wound healing biomarkers viz., interleukin 6(IL-6), interleukin β (IL-β), insulin-like growth factor tyrosine kinase receptor (IGF-1R) and transformation growth factor β (TGF-β). Out of 10 selected compounds, three compounds (mesalazine, catechin and piperazine) present in the aqueous crude extract of *Typha domingensis* Pers. inflorescence had a strong binding affinity with good Glide score value and docking energy against selected receptors that are involved in different stages of wound healing. The cell migration/scratch assay study which was carried out to find the efficacy of these three compounds in an in vitro system. The results of scratch assay revealed that compared to control (52.5%), *T. domingensis* Pers. inflorescence crude extract showed maximum wound healing potential (80%) followed by Catechin (66.8%) Mesalazine (58.3%) and Piperazine (51.2%).

Findings of this combined study with the in silico and in vitro approach, provide promising results. However, in vivo studies are needed to ascertain these compounds for their effective therapeutic targets and for the management of various types of wounds and wound related problems.

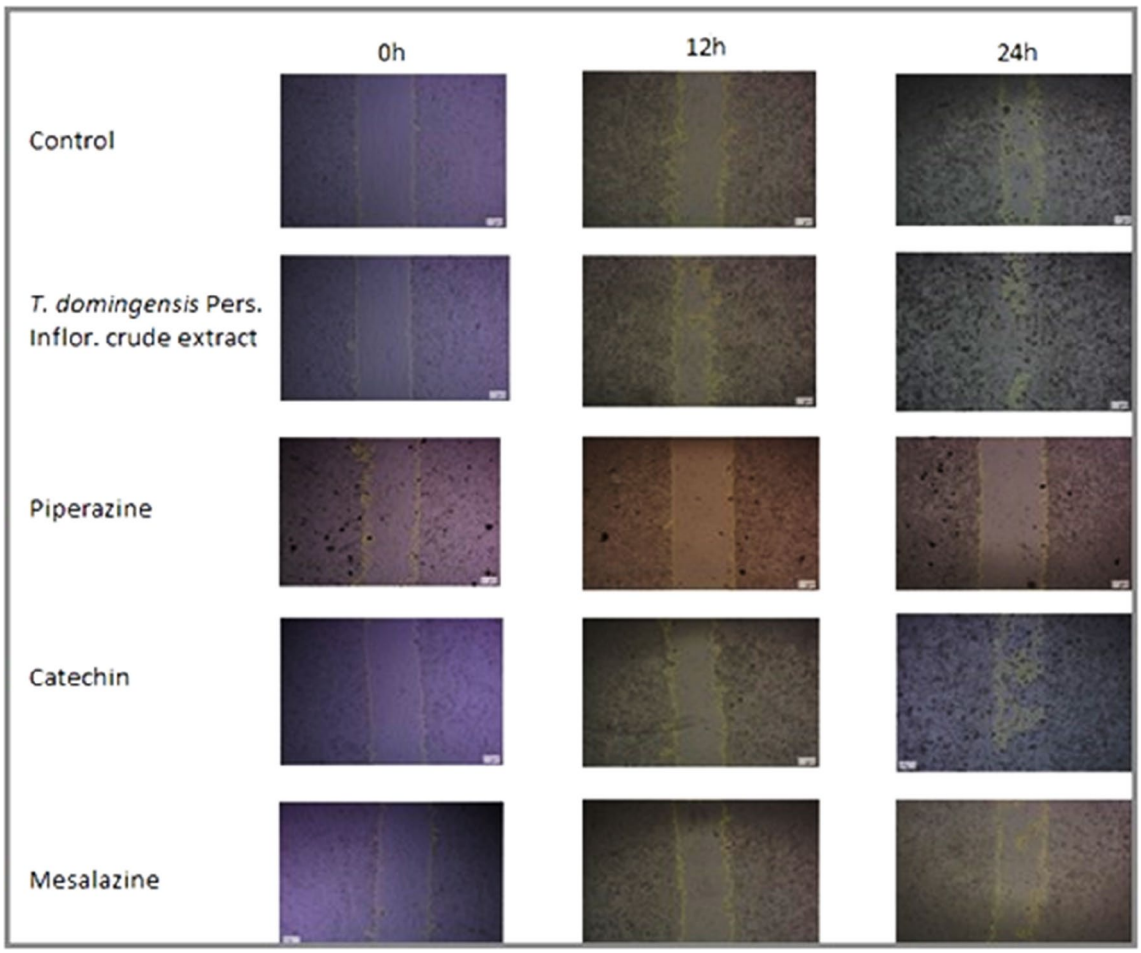

Fig. 7 Effect of *Typha domingensis* Pers. crude extract and synthetic compounds on cell migration assay on 3T3 mouse fibroblast cell line

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Data availability All data generated or analyzed during this study are included in this article.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent to publish Not applicable.

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