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Original Research Article

Discerning the regulated wound healing potential of *Ocimum americanum* by probing the rosmarinic acid content-a paradigm on zebrafish caudal fin regeneration

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ABSTRACT

Acute traumatic wounds create a serious impact on pathology and impede recovery management. This work emphasis on the wound healing potential of *Ocimum americanum* extract by caudal regeneration studies in zebrafish model. Molecular docking studies exhibited high binding energy (-8.8 kcal/mol) and an inhibitory effect was conferred between the plant's standard compound rosmarinic acid against gelatinase. To evaluate its efficacy the plant extract is queried for the presence of phenolic components analogous to rosmarinic acid. The FTIR studies showed similar functional groups with the incidence of phenolic contents and the HPLC assay validated its presence by depicting a communal peak at 4.19 min. *In vivo* studies authenticated the LC_{50} value at an optimum concentration of 135.51 ± 0.01 mg/L. The caudal fins were excised to assay regeneration capacity and histopathology sections augmented restoration. Together these results conclude the wound healing capability *O. americanam* with the probability to regenerate caudal fins and reparation.

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1. Introduction

The worldwide epidemic perspective of wound healing is concerned as a difficult obligation. Healing acute and chronic wounds remains a challenge because it has failed to follow the orderly process of auto recovery (Yao et al., 2020). The incidence of these wounds is interposed with several ailments such as trauma, diabetes, vascular disease, infection, allergy, etc. (Jarbrink et al., 2016). Prolonged hospitalization is a vulnerable reason for these types of wounds where, 70% relapse and 34% recur with infection. Globally 2% of adult and old patients are experiencing a morbid risk due to aging as an impairing factor (Powers et al., 2016). A better understanding of the effect of these features on repair may lead to therapeutics that improve wound healing and resolve impaired wounds (Gorski et al., 2020). Gelatinase are extracellular matrix metalloproteinases (MMPs) with a potential role in tissue repairing and remodeling (Neely et al., 2000). But many factors can interfere and hinder restoration. Hence, there is a need to disseminate remedies that optimistically regulate these proteins and improve patient care (Okur et al., 2019; Baron et al., 2020). Traditional medicines have established as a thriving resource in wound care management and authenticated with excellent outcomes. Certainly, some plant derived compounds and extracts could deliver novel reagents and therapies for today's therapeutic challenges (Chambliss et al., 2001; Elmastaş et al., 2018; Erenler et al., 2018). Rosmarinic acid (α-*O*-caffeoyl-3,4-dihydroxyphenyllacticacid) is one such natural phenylpropanoid (phenolic compound) instigated from *Rosmarinus officinalis* L (called rosemary) which possesses exclusive wound healing efficacy (Wani et al., 2019; Yilanci et al., 2020). Myriad of natural and their derived products are concealed, undefined and unexplored. Such adjunct compounds should be investigated as lead drug moieties in contemporary therapeutic research. Hence this work lays a pedal

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stone to probe the constituents of rosmarinic acid from *Ocimum americanum* a commonly grown medicinal plant in South India. This plant is also known as lime hairy or "hoary /lemon basil", is an annual herb and despite the deceptive name, it is native to Africa, the Indian Subcontinent, China, and Southeast Asia.

The genus *Ocimum* is a miscellany group that constitutes significant medicinal plants with a rich source of aromatic oils. In particular, *O. americanum* possesses the foremost phytocompounds such as *trans*-β-ocimene, β-bisabolene, (*E*)-γ-bisabolene, methyl chavicol and 1,8-cineole which are considered as an essential oil (Tewari et al., 2013). Considering this as a vital factor the oil like composition (Erenler et al., 2016) inherited from the plant is corroborated with the potentials of rosmarinic acid. Furthermore, the antioxidant (Koysu et al., 2019) and cytotoxic capabilities were considered as an advantage to treat wounds and could serve as a good source for crude drug preparation. Hence the study is planned to target the wound healing and caudal regeneration on zebrafish models induced with tail excision. There is a robust fin regeneration in the zebrafish and hence the efficacy of the plant is validated based on its time of growth which is compared on other normal species. The regenerative output of caudal fin in zebrafish is unlimited which is used as a significant augmentation (Richardson et al., 2016). This preliminary work validates the wound restoration efficacy of *Ocimum americanum* on par with the rosmarinic acid extract.

2. Experimental

2.1. Chemicals and reagents

All chemicals and reagents used in this study were of analytical grade, purchased from Hi-Media Laboratories Pvt. Ltd., India. Rosmarinic acid (96%) was obtained from Sigma-Aldrich, India.

2.2. *In Silico* analysis

The 3D structure of gelatinase (Target protein) a catalytic domain (1QIB) was downloaded from PDB and its physiochemical properties were predicted using ProtParam (https://web.expasy.org/protparam/) which is depicted in supplementary Table S1. The phytoconstituents (ligands) were obtained from PubChem with specific ID numbers (Linalool-CID: 6549; Eugenol-CID: 3314; β-bisabolene-CID: 10104370; methyl chavicol-66957732; *E*-γ-bisabolene-CID: 5352437; Rosmarinic Acid-CID: 5315615).Their molecular formula and canonical SMILES are presented in the Table S2.The molecular properties of the ligands and their structures were determined utilizing Medchem designer. The drug-likeness similarity of the chose ligands was assessed by Lipinski's rule of five (Lipinski et al., 2001). The target protein structure was docked against the ligands using AutoDock Tools 1.5.6. (Morris et al., 2009). The proteins were prepared as per the standard procedure. The water molecules were removed, the polar hydrogens were added and any presence of non-protein heteroatom groups was removed followed by assigning gasteiger charges. For all the small molecule structures, all bonds were made rotatable. The grid was generated at the ATP binding site with the maximum grid spacing of 1Å. The selected ligands were docked into the catalytic centre of the kinases using Auto Dock Tools 1.5.6 with the default exhaustiveness of 8. Since AutoDock Tools 1.5.6. calculates the inhibitory constant (K_i), automatically, using the formula

$$K_i = \exp\left(\frac{\text{deltaG}}{RT}\right) \quad \text{(Cosconati et al., 2010)} \quad \text{(Eqn. 1)}$$

Where, gas constant, R = 1.9871917 cal/K.mol and absolute temperature, T = 298.15 K. The resulting affinity and K_i were compared and assessed. The 2D poses of the best hits of each of the compounds docked with the target protein(1QIB) were generated using Accelrys Discovery Studio Visualizer 2.5(Visualiser) and Ligplot analysis.

2.3. Collection and authentication of plant

The plant samples of *Ocimum americanum* (leaves) were collected from the rural skirts of Dindigul district (Pudur), Tamil Nadu, India. The plant material was mounted in herbarium sheets for taxonomical identification based on their morphological features. The plant was identified (RM001) and authenticated by Rapinat Herbarium and Centre for Molecular Informatics, St.Joseph's College, Tiruchirappalli Tamil Nadu, India.

2.4. Plant extraction

The dried leaves of the plant were considered as the test sample and water was used as a solvent for the extraction. Five grams of dried leaves were soaked in 100 mL of boiling water for 30 min and kept for continuous agitation for 24 hrs (120 rev/min). The contents were filtered and then lyophilized (GSD-50). The extract was formulated to a semisolid form and the solvent was removed by sturdy evaporation at 60 °C using rotary vacuum evaporator (2199A-H). The standard of rosmarinic acid is prepared by dissolving 1 mg/10 mL of methanol solvent and used as a reference solution of 0.1 mg/mL. The extracts were stored in a freezer at -40 °C for further analysis.

2.5. Probing functional identity using FTIR and HPLC analysis

The functional groups present in the leaf extract were analysed through FTIR (FTIR-8400S, Shimadzu, Japan with 32 bit operating software) using rosmarinic extract as reference and the spectra were recorded from 400 to 4000 cm^{-1} with the resolution of 4 cm^{-1}. Furthermore, the phytochemical profiles of the test (plant extract) and standard (rosmarinic acid) sample were comparatively assayed using high performance liquid chromatography (HPLC), model Shimadzu, Kyoto, Japan with a Prominence auto sampler (SIL-20A), equipped with Shimadzu LC-20 AT reciprocating pumps. The analysis was performed with an integrator

(CBM 20A), a UV-VIS detector DAD (diode: SPD-M20A), and the Software LC solution 1.22 SP1. The separation was based on reverse phase with ODS-C18 column (450 mm x 4.6. i.d). The sample was filtered with 0.45 µm pore size membrane. Isocratic system was used with mobile phase consisting of 80% methanol as solvent (a) and 20% (water with 0.1% acetic acid) as solvent (b). The flow rate and injection volume was 1 mL min^{-1} and 10 µL, respectively. Signals were detected at 280 nm where phenolic compounds exhibit maximum absorption

2.6. Wound healing and caudal regeneration studies

2.6.1. Rearing and maintenance of animals

Adult zebrafishes were obtained from a commercial dealer and 10-15 fishes were kept in 5 L acrylic tank with the following conditions; 28.5 °C, with a 14/10 h light/dark cycle. The zebrafish were 2-3 months old, weighed 0.5 ± 0.2 g and their mean length was 30 ± 4 mm. The fishes were fed three times per day, with commercially available dry fish food. The fishes were allowed to acclimatize to the laboratory conditions for two weeks before the experiment. Fishes weighing close to 0.4 g were selected for the experiment.

2.6.2. Design of the acute toxicity (LC_{50} value) test

The 96h acute toxicity of the test and standard were conducted on zebra fish. The plant extract was dried using rotary evaporator (Yamato RE-201-100C) and further liquified with water. The rosmarinic acid powder was dissolved with water and was prepared as aqueous extract. The test and standard extracts were prepared as stock solutions and diluted further as desired. All the *in vivo* studies were investigated by a semi-static test performed according to the guideline 203 of OECD (1992). Every control (Negative control-without test and standard exposed to saline water) and treatment group were replicated three times and each repeat consisted of five adult zebrafish and 4L exposure solution. Moreover, the entire exposure solutions were renewed every 2 days. The pH, temperature and dissolved oxygen concentration of the exposure solutions were the same as the water used in the acclimation procedure. Other feeding conditions are consistent with the conditions in the previous domestication process. Zebrafish were fed with commercial dry diet once daily and stopped feeding at 24 h prior to the acute toxicity test. During the test period, the dead fish and faeces were removed in a timely way to avoid interference. Estimation of LC_{50} values were done by direct interpolation method in which two exploratory tests and one definitive test was carried out. The mortality was recorded after 24, 48, 72 and 96 hrs exposure period. The concentrations from definitive test were used to evaluate the LC_{50} value by plotting a dose response curve between % mortalities and concentrations of toxicant. For statistical analysis, the obtained values of definitive test were converted into log concentrations, correct% mortalities and probit using Finney's Table (1971).

2.6.3. Tail wounding assay and caudal fin regeneration studies

2.6.3.1. Preparation of the wound model

Caudal fin amputation was performed after determining the lethal dosage value. The fishes were grouped into control and two experimental groups (aqueous and rosmarinic acid extract), each group having 30 fishes including triplicates. The fishes were anesthetized with tricaine for three and a half minutes. The caudal fin was transfected 5 mm from the posterior end with a sterile scalpel to the first lepidotrichia branching point. The fishes were observed on 3-, 5- and 7-days post-wounding for fin regeneration. The fin growth was measured and the results were photographed during the pre- and post-amputation under a dissecting microscope equipped with a camera.

2.6.3.2. Histopathological examination

For histological examination the excised caudal fin segments were fixed in 4% paraformaldehyde in phosphate buffered saline at room temperature overnight. Then the intestine samples were dehydrated in 70% ethanol fixed in par formaldehyde, cleared in xylene and embedded in a section of 7 µm thickness were prepared from paraffin blocks using a rotary microtome and then stained with hematoxylin-eosin. Histopathological changes were examined under a microscope.

2.7. Statistical analysis

Results were presented as mean ± standard deviation (SD). The standard deviation was presented by the error bar. SPSS 22.0 software (SPSS Inc., USA) was utilized to measure the 96 h LC_{50} value via probit analysis and to evaluate the data of subacute toxicity test via a one-way analysis of variance (ANOVA). The statistical difference between the treatment and control groups at the same time point was validated using the least significant difference (LSD) test and $p < 0.05$ was regarded as statistically significant.

3. Results and Discussion

Medicinal plants are sanctified to have immense ethnopharmacological, therapeutic and commercial importance and has been used in traditional medicines (Mohammadhosseini, et al. 2021a; Mohammadhosseini, et al. 2021b; Nahar et al., 2021). There are innumerable herbal plants which are sourced as lead compounds for drug development for various ailments. Despite incredible advances in modern science, technology and allopathic medicine there is a huge lack in providing quality healthcare to all. Herbal medicine considered as a major healthcare provider around the globe particularly in rural and remote areas (Olaoluwa et al., 2022). A large section of people depends on such medicine for their primary healthcare mainly in underdeveloped or developing countries. Indian traditional medicinal system like Ayurveda, Siddha and Unani has a very rich history of their effectiveness; modern research

also acknowledged the importance of such medicine. Indian traditional medicine or medicinal plants are also considered as a vital source of new drug. Mainstreaming of such medicine is important for the people. This study intends to discern an elucidation to wound healing related issues through an herbal strategy by assaying the therapeutic efficacy of *Ocimum americanum*. This plant has been profiled with a high medicinal significance and has been traditionally used in treating skin disease It is also applied to perilous wounds and burns that are challenging to heal (Zhou et al., 2013).

3.1. Inhibition of matrix metalloproteinase by *in silico* studies

The molecular properties of the chosen ligands obtained from Medchem designer reveal that the calculated properties of rosmarinic acid have significant therapeutic values [0] that resemble with zero infringement of Lipinski's standard (Table S3). The bioactivity scores of the test compounds were determined by the Molinspiration Cheminformatics tool and the information is provided in Table S4. It is archived that, if the bioactivity score is more than 0, the particles have better biological activity. The binding affinities of all six essential oil constituted compounds were verified by molecular docking against the target protein gelatinase is reported in Table 1. The grading of the docking score apparently demonstrates the high binding affinity of rosmarinic acid (-8.8 kcal/mol) followed by linalool (-8.0 kcal/mol). The best docking pose of the ligands with the crystal structure of gelatinase (a catalytic domain 1QIB) binding sites is as shown in Fig. 1a. A maximum of seven hydrogen bond interactions were exhibited by rosmarinic acid (thr 227, ala 22, leu 218, ala 167, his 211, leu 164, glu 202, his 201), where linalool and eugenol (Fig. 1b) showed one each at thr 227. Results from molecular docking studies revealed that rosmarinic acid had several folds lower Ki (354.42 nM) and a lower ΔG of -8.8 kcal/mol towards (a catalytic domain 1QIB)- when compared to other ligands.

Wound management is a significant progression that requires a targeted mechanism devoid of adverse effects. Primordially, herbal medicines have been used to accelerate wound healing and *Ocimum americanum*, is one such potential plant encompassing high amount of aromatic and essential oils (Sutili et al., 2016) where, rosmarinic acid is an essential constituent. The *in silico* molecular docking studies prompted the binding affinity of the foremost essential oil constituents of the plant towards gelatinase (Matrix metalloproteinase-MMP2). MMP2 are class of matrix degrading enzymes that plays a vital role in wound healing and its expression has been upregulated in subepidermal layer of skin injury (Caley et al., 2015). Yet, impeding its action is considered as a valuable approach for treating chronic ailments like diabetes mellitus, peripheral arterial disease, immune deficiencies and cardiopulmonary obstructions. Rosmarinic acid excelled with a finest minimum energy score and confined a high inhibitory potential determining a therapeutic efficacy in wound management.

3.2. Probing of phenolic derivatives from the plant extract

3.2.1. FTIR and HPLC

FTIR measurements were carried out to identify the relative functional groups present both in the plant extract and rosmarinic acid. The comparative FTIR spectrum profiles are illustrated in Fig. 2. The plant extract exhibited distinct peaks associated with the standard. The band at 3448 cm^{-1} corresponds to O-H stretching, H-bonded alcohols and phenols. The peak at 2965 cm^{-1}; 2926 cm^{-1} associated to C-H stretch. A characteristic peak at 1721 cm^{-1} relates to the ester stretching C=O which is conjugated with a double bond. The assignment at 1633 cm^{-1} parallels N-H bends primary amines. The peak at 1377 cm^{-1} refers to C-N stretching of aromatic amine group and the bands observed at 1274, 1231, 1020, 595 cm^{-1} indicate the presence of alkanes, ethers and Halides. HPLC assay was performed to detect the content of rosmarinic acid and its related phenolic derivatives in *Ocimum americanum*. The related chromatographic peaks and their retention time corroborated the presence of phenolic derivatives of plant extract on par with rosmarinic acid. The plant sample (Fig. 3a) illustrates two characteristic values with a heightened sharp peak at 4.194 min regulatory (Rosmarinicacidhexoside) and another definite peak with a retention time of 6.628 min which denotes Rosmarinicacid sulphated II isomer. These values were evaluated with the reference sample and substantial peaks at 3.475 min and 9.747 min represented rosmarinic acid and caffeicacid. A communal peak (Fig. 3b) was obtained at a retention time of 4.19 min which refers to the constituents of phenol derivates such as rosmarinic acid. Rosmarinic acid is an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid (Gamaro et al., 2011). The time lapse of retention peak was immediate, and this is due to the interrelation between the phenolic compounds of rosmarinic acid and an ester of caffeic acid. The wound healing potency of traditional herbs is attributed due to the synergistic role of the phytoconstituents (Genc et al., 2020). The presence of polyphenols and rosmarinic acid derivatives in aqueous extract is authenticated in the study by FTIR and HPLC studies. The distinct bands at 1450 and 1633 cm^{-1} elucidates the stretching of aromatic ring (Coates 2000; Stehfest et al., 2004). Further, the overlaying of phenol stretching (O-H and the carboxylic group) at > 3000 cm^{-1} along with the peak at 1377 cm^{-1} evidence for the phenolic compounds. The assemblage of rosmarinic acid, chlorogenic acid, caffeic acid, hydroxyl cinnamic derivatives is categorized as significant polyphenols that hoist the medicinal potential of the plant. In particular, *O. americanum* constitutes an elevated index related to phenolic derivates, where rosmarinic acid, (an ester of caffeic acid and 3.4-dihydroxyphenyllatic acid) is considered as a significant compound (Rady and Nazif 2005). The HPLC analysis of crude plant extract exemplified rosmarinicacid hexoside with a heightened peak encompassing increased retention time and was preceded by rosmarinicacid sulphated II isomer (Ozarowski et al., 2016). Thus, the communal peals

Table 1
Molecular docking analysis with the selected compounds with the crystal structure of gelatinase a catalytic domain (1QIB).

Name of the Compound	Binding affinity (Kcal/mol)	Inhibitory constant (Ki)	Hydrogen bonding interaction
Linalool	-8	39.99 µM	THR 227...O
Eugenol	-6.2	28.53 µM	THR 227....H
β-Bisabolene	-6	1.37 µM	-
Methyl chavicol	-6.2	28.53 µM	-
E-(Y)-Bisabolene	-7.5	3.18 µM	-
Rosmarinic acid	-8.8	354.42 nM	ALA 220...O,LEU 218...H, ALA 167...H,HIS 211...O, LEU 164...O, GLU 202...H, HIS 201...Pi-cation

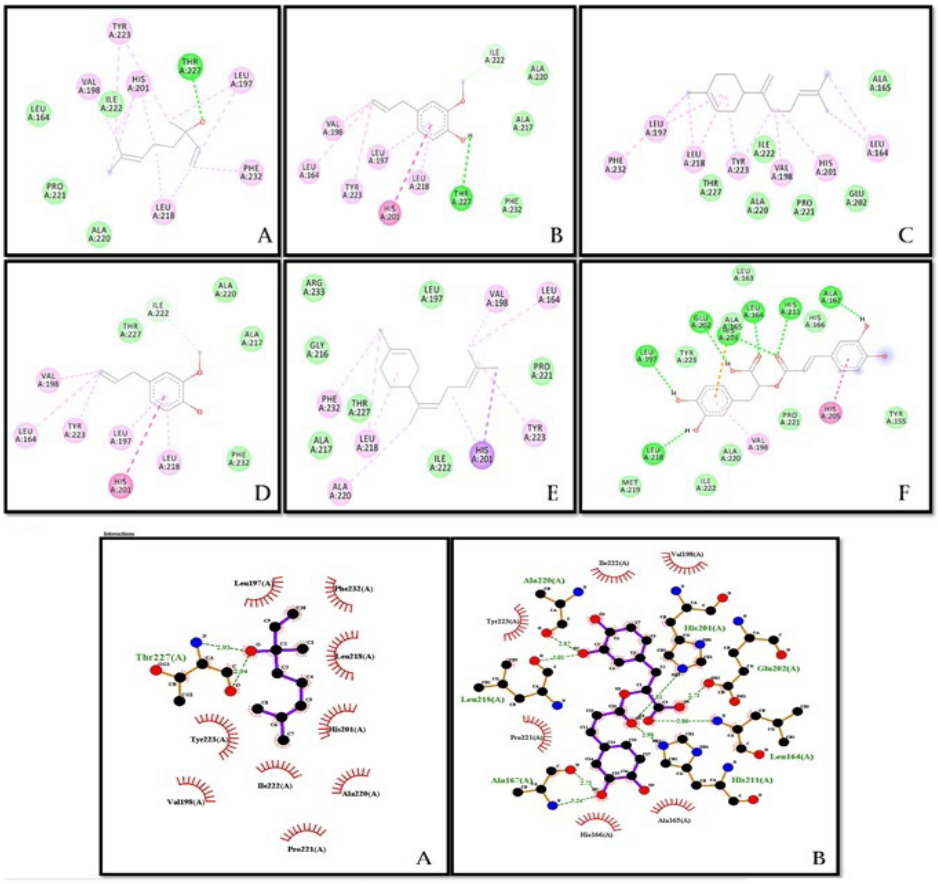

Fig. 1 a. Ligand binding residues with the target protein matrixmetalloproteinase-2(Gelatinase) b. Lig plot illustration of molecular interaction of A. Linalool and B. Rosmarinic acid.

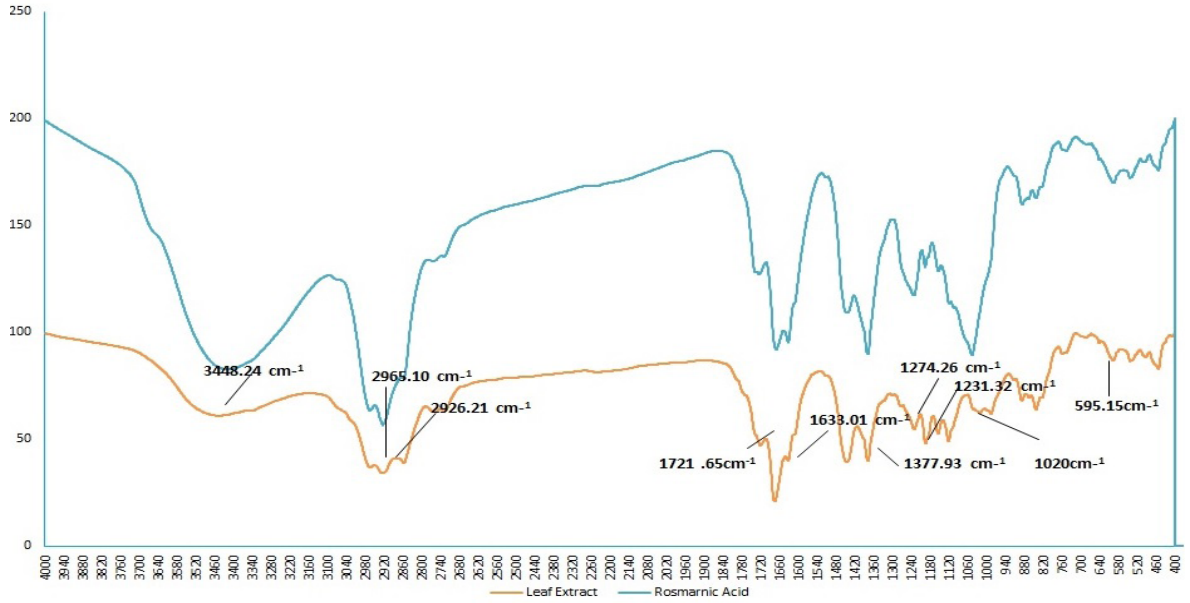

Fig. 2. The FTIR spectra of test and reference samples.

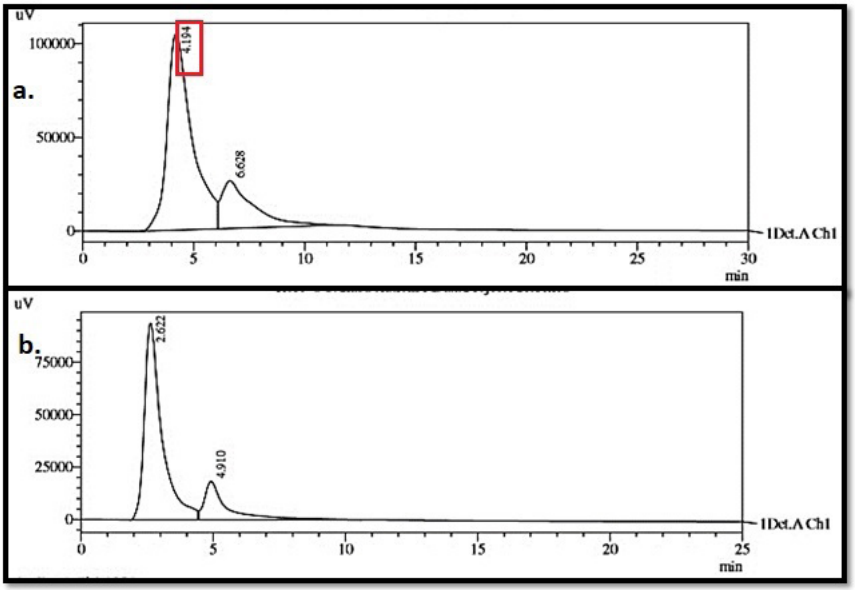

Fig. 3. Chromatograms of aqueous leaf extract of a. *Ocimum americanum* b. Rosmarinic acid extract.

validate the presence of phenolic derivatives that were analogous in both test and reference samples.

3.3. Acute toxicity (LC_{50}) studies

Estimation of LC_{50} values was done by the direct interpolation method in which two exploratory tests and one definitive test were carried out. During these tests, the mortality was recorded after 24, 48, 72 and 96 hrs exposure. After 24 hrs of initial exploratory test for the plant extract, 100% mortality was obtained at 0.25 g/L while all fishes were remained alive at 0.002 g/L. In the 2nd exploratory test, only the higher concentrateion 0.1g/L exhibited mortality of 10% whereas the lower concentrations (0.01-0.05 g/L) did not show death rates after 96 hrs.On the basis of 2nd exploratory test six concentrations (0.008, 0.035, 0.080, 0.120, 0.180. and 0.240 g/L) were selected for definitive test and mortality rate was observes in 0.120, 0.180. and 0.240 g/L (Table S5). A dose response curves were plotted between concentrations and % mortalities (Fig. 4 a) and LC_{50} values were obtained by drawing a perpendicular on the curve at 50% mortality with a concentration of 0.170 g/L respectively after all exposure period (Table S6). The different concentrations of the plant extract were expressed (Fig. 4 b) as log values and the probit scores

were calculated and was found to be 135.51 mg/L for the 96 hrs of exposure. Establishing herbal medicines as therapeutic leads requires the evaluation of their toxicity. Due to ethical considerations and constraints, the use of higher vertebrates for toxicity testing has become more combative. Small animal studies using zebrafish render an alternative to mammalian animal models and evaluate various human ailments (Bambino and Chu 2017). Apparently, the mortality of fish increased ensuing an acute toxicity effect which was directly proportional to the concentration of plant extract. There was a substantial dose-response relationship between the mortality rate and the logarithm of experimental concentration with time.

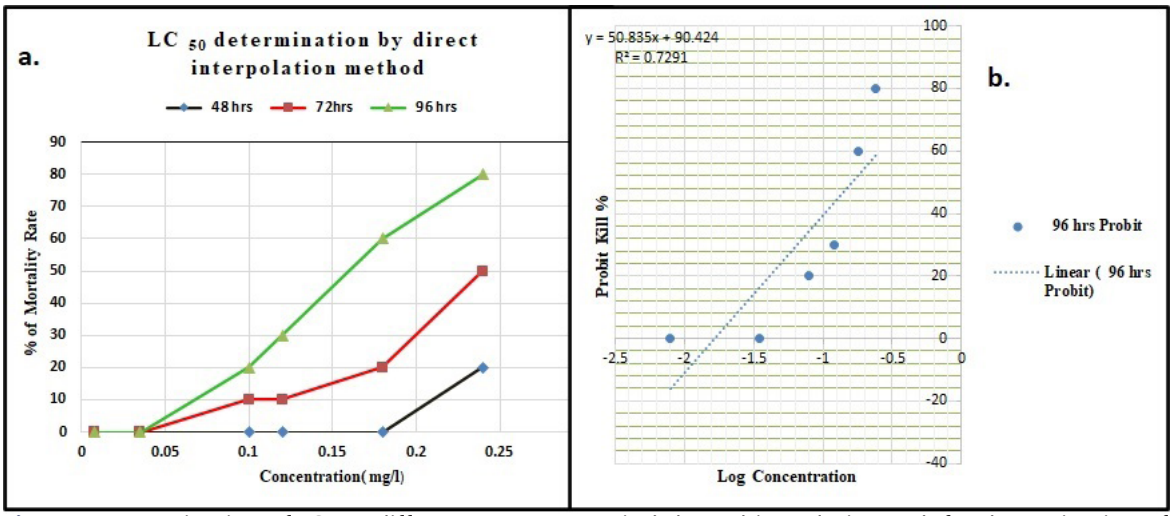

Fig. 4 a. Determination of LC_{50} at different exposure periods b. Probit analysis graph for determination of LC_{50} -96 hrs exposure.

3.4. Determination of wound healing activity

3.4.1. Caudal fin regeneration studies

The fin growth measurement on days 1, 3, 5 and 7 was compared with the control. The fishes treated with the leaf aqueous extract (*Ocimum americanum)* restored regeneration on par to those treated with the rosmarinic acid with the rate of regeneration increasing with concentration (Fig. 5). The fishes treated with 160 µL of the aqueous extract showed maximum fin regeneration (0.9 mm) on day 7 compared to the control fishes. The zebrafishes were very subtle to rosmarinic acid and at higher concentrations they were fatal. The minimum concentration responded and reinstated fin restoration with a maximum length of 1.2 mm at 20 µL. Though the fin regeneration of *O. americanum* extracts bestowed lower measurement in the length it established a healthy restoration with an organized process devoid of adverse effects (abnormal morphology and impaired formation) and fatality. An adult Zebrafish is capable of regenerating its complete set of fins (anal; caudal; dorsal; pectoral; pelvic). The caudal fin is primarily focused onto assay the proportion of restoration and generally the morphology is replaced within 1-2 weeks of excision (Poss et al., 2003). There was an increased fin growth in both rosmarnic acid and plant extract. Where, the test samples induced a homogeneity in all the fishes when compared to those exposed to rosmarnic acid (distorted appearance).

3.4.2. Histopathological examination

The histopathological studies illustrate (Fig. 6) the tissue regeneration process of zebrafish.The neutrophil (N) population and the formation regeneration epithelium (E) substantiates the rejuvenation of tail growth and formation.The treated experimental sets (Rosmarinic acid A and plant extract C) showed a thicker regeneration pattern of the epithelium than the untreated (B) which showed a distorted appearance. The rate of neutrophil proliferation was distinct in both the treated samples and plant extract highlighted more neutrophils. The regulated growth and morphogenesis of fin regeneration are associated with the blastema formation by the mesenchyme-derived progenitor cells (Sousa et al., 2011) which was visualized by the thickening of the epidermis in the histopathological examination. The wound healing potency of traditional herbs is attributed due to the synergistic role of the phytoconstituents. The anti-microbial properties of flavonoids, phenols and terpenoids have the affinity to induce inflammatory factors and prolongs the process which results in evasion from microorganism at the wound site (Gorski et al., 2020). Various glycosides have been reported to contribute to the anti-inflammatory and wound healing activities of therapeutic preparations (Korkina et al., 2007). The insightful antioxidant potential of *Ocimum americanum* chelates the free radicals and reactive oxygen species. Thereby, the herbal extract can promote wound contraction and

Fig. 5. Zebra fish caudal fin transection and regeneration (A) Normal fish with an uncut tail fish; (B) Fish after caudal fin transection; (C) 10 µL (D) 20 µL lemon balm rosmarinic extract treated fish on day 7 post-transection (Impaired tail formation); (E) 160 µL aqueous extract treated fish on day 7 post-transection. Scale bar = 19 mm.

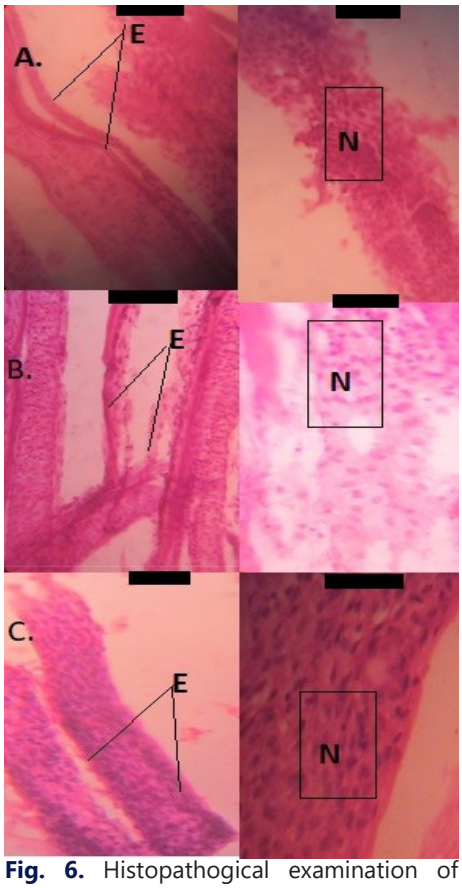

Fig. 6. Histopathogical examination of wound site illustrating the Epithelium (E) restoration and Neutrophil population a. Treated -Rosmarinic acid ×100 scale bar 30 µm B. Untreated×100 scale bar 30 µm C. Treated -plant extract analyzed using H&E staining. ×100 scale bar 30 µm.

aggregates the formation of capillary vessels along with fibroblasts. Invasion of acute inflammation is due to the expression of the pro-inflammatory signals that are induced by chemokines. Polyphenols possess the mechanism of stimulating chemokines and are widely used in wound dressings (Moldovan et al., 2011) and the study authenticates its presence in the aqueous extract. Thus, the phenolic derivatives along with the resourceful phytoconstituents heighten the wound healing property of *Ocimum americanum* and establish it as a significant medicinal herb.

4. Concluding remarks

This work enumerates the efficacy of *Ocimum americanum* as a therapeutic lead in wound healing and augments its sustenance with resourceful phytocompounds like rosmarinic acid. The ingenious phytoconstituents of *Ocimum americanum* embellished with the resourceful phenolic derivatives enhances its wound healing property and institutes it as a momentous therapeutic lead. The study pilots the probing of rosmarinic acid like phenolic derivatives, and thus strategies compound isolation and quantification on higher animal models. Conversely, the tail regeneration studies intensify the wound restoration capacity of the plant in a healthy manner devoid of abrasions and irregular morphology. The adult zebrafish fin regeneration system is a modest and incredible alternative model to reveal, the wound healing ability of the plant extract. The molecular facts related to prompt wound healing and regeneration are to be explored with advanced technology in the established model and it is considered an imperative requisite in futuristic studies.

Conflict of interest

The authors declare that there is no conflict of interest.

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