Wound healing activity of methanolic and aqueous extracts of *Plagiochila beddomei* Steph. thallus in rat model

G S Manoj & K Murugan*
Plant Biochemistry and Molecular Biology Laboratory, Department of Botany, University College, Thiruvananthapuram, 695 034, India

Received 28 November 2011; revised 24 April 2012

Wound healing occurs as a fundamental response to tissue injury. Polyphenols are considered to be principal constituents to promote wound healing. *Plagiochila beddomei* was applied clinically by Paliyar tribes of Madurai district to treat intractable wounds. To validate the ethnotherapeutic claims of the plant in skin diseases, the effect of methanolic and aqueous extracts from *P. beddomei* on wound healing as well as angiogenesis was studied. On 7th and 10th day after excision wounds creation, the percent wound contraction of the extracts group was higher than that of Madecassol® (reference drug) group. On 3rd, 7th and 10th day after wounds creation, the wound healing quality of the extracts group was better than that of Madecassol® group on terms of granulation formation and collagen organization. On 3rd day after wounds creation, the micro vessel density and vascular endothelial growth factor expression of methanolic extracts group was higher than that of Madecassol® group. Phytochemical analysis of the extracts showed the presence of flavonoids, saponins, tannins and phenols. The results show that *P. beddomei* extract has potent wound healing property probably resulting from the remarkable angiogenic activity.

**Keywords:** Angiogenic, Excision wound, Incision wound, *Plagiochila beddomei*, Secondary metabolites, Wound healing

Wound is defined as the disruption of the cellular and anatomic discontinuity of a tissue. Wound healing consists of an orderly progression of events that reestablish the integrity of the damaged tissue. Research on wound healing drugs is a developing area in modern biomedical sciences. Many of the synthetic drugs currently used for the treatment of wounds are not only expensive but also pose problems such as allergy, drug resistance etc. and this situation have forced the scientists to seek alternative drugs. A substantial number of drugs are developed from plants, minerals and animals and are described in the Ayurveda for their wound healing properties under the term Vranaropaka. The majority of these involve the isolation of the active ingredient found in a particular medicinal plant and its subsequent modification.

*Plagiochila beddomei* Steph. is widely distributed species in different parts of India. Ethnomedicinally, it is used by Paliyar tribe in Madurai district of Tamil Nadu state of India for treating skin diseases in the form of paste. The fresh material is applied externally for the treatment of burns, boils and blisters on the body. It is also applied for the treatment of skin eruption caused due to bright sun in summer. Most often the use of plant is merely based on folklore or tribal knowledge without any scientific evidence of efficacy and the effective compounds are unknown. The present study has been designed to evaluate effect of methanolic and aqueous extracts of *Plagiochila beddomei* Steph. on wound healing, angiogenesis and vascular endothelial growth factor A (VEGFA) expression in rat wound model.

**Materials and Methods**

*Materials—* Fresh thallus of *Plagiochila beddomei* was collected, from Westernghats region of Nilgiri hills of Tamil Nadu, India. The plants were identified by comparing with the authenticated specimen and voucher specimens were deposited in the institute herbaria.

*Experimental animals—* Sprague Dawley rats of either sex (160-180 g) purchased from the animal house of Mahaveera Enterprises, Hyderabad, India were maintained under standard laboratory condition (25 ± 2 °C, 55 ± 5% RH), and 12:12 h L:D cycles for about 7 days prior to experimentation. The animals were housed three per cage of same sex in

* Correspondent author
Telephone: 9447077895
E-mail: harimurukan@gmail.com
polypropylene cages provided with sterile bedding of paddy husk for 1 week before the experimentation as acclimatization period. Pellet chew feed standard diet under good management conditions and water *ad libitum* was provided to the animals. After wound creation, one animal per cage was maintained. All studies were conducted in accordance with the protocol of National Institute of Health. The study was undertaken after obtaining approval from Departmental Ethical and biodiversity right committee (Institutional Animal Ethics Committee (IAEC) approval letter NO. IAEC/UC/11/2011-2012, 08 January 2011).

**Preparation of extract**—Fresh thallus was weighed, chopped, dried in shade and powdered mechanically. About 100 g powder was subjected to soxhlet hot continuous extraction with methanol at 78 °C followed by distilled water at 100 °C for about 48 h. The methanolic and aqueous extracts were filtered and concentrated *in vacuo* gave a residue 4.9 g and 3.86 g respectively.

**Preliminary phytochemical analysis**—The presence and absence of different phytochemical constituents, viz., phenols, flavonoids, carbohydrates, glycoproteins, alkaloids, tannins, sterols and triterpenes were recorded.

**Thin layer chromatography of extracts**—Methanolic and aqueous extracts of *P. beddomei* were subjected to thin layer chromatographic studies, to find out the probable compounds present in them.

**Preparation of the plates**—The adsorbent used for thin layer chromatography was silica gel G. The precoated TLC plates (Merk, Germany) were heated in an oven for 30 min at 110 °C for activation. The test sample (5 mL; 1 mg/mL in alcohol) was applied in the form of bands using LINOMAT IV applicator. Four bands using different volumes in increasing order were applied.

**Developing solvent system**—Many developing solvent systems were tried, but the satisfactory resolution was obtained in the solvent systems mentioned below. The solvent system was ethyl acetate:methanol:water (5:1:1:1). After development of plates, they were air-dried and numbers of spots were noted and *Rf* values were calculated. Spots were visualized by spraying with various spraying reagents to find different compounds present in the extract. Aluminum chloride (10 % in methanol) for flavonoids, ninhydrin solution (2 % in methanol) for amino acids, amines and amino sugars, ferric chloride reagent, sulphuric acid reagent (5% in methanol).

**Incision wound model**—The rats were anaesthetized with 0.15 cc Ketalar® injection and dorsal hair of the rats were shaved with electric clipper. Two linear-paravertebral incisions of 5 cm length were made with a sterile surgical blade through the full thickness of the skin at the distance of 1.5 cm from the midline of each side of the depilated back of the rat. The wounds were closed with three surgical interrupted sutures of 0.5 cm apart. All the sutures used in the experiments were non-absorbable braided non-capillary and siliconized. The animals were divided into following four major groups of thirty rats each; Gr. I was served as control, Gr. II received methanolic extract topically, Gr. III received aqueous extract and Gr. IV received reference standard drug (Madecassol®). Test samples were prepared in an ointment base (vehicle) consisting of glycol stearate, 1, 2 propylene glycol, liquid paraffin (3:6:1) in 1% concentration. Test ointment (0.5 g of each) was applied topically on the wounded site immediately after wound was created by a surgical blade. Reference drug group of animals were treated with 0.5 g Madcassol®. Madecassol® (cream 1%) contains Centella asiatica; reconstituted dry extract containing asiaticoside (40%) and madecassic and asiatic acids (60%). All the drugs were given daily till epithelialization. All the sutures were removed on the 9th post wound day. On 1, 3, 7, 10 and 14th days the animals were sacrificed by administering intravenous thiopentone sodium (20 mg / kg body weight).

**Excision wound model**—An impression was made on the dorsal thoracic region 1 cm away from vertebral column and 5 cm away from ear using a round seal of 2.5 cm diameter on the anaesthetized rat. The skin of impressed area was excised to the full thickness of the skin at the distance of 1.5 cm from the midline of each side of the depilated back of the rat. The skin of impressed area was excised to the full thickness of the skin at the distance of 1.5 cm from the midline of each side of the depilated back of the rat. The skin of impressed area was excised to the full thickness of the skin at the distance of 1.5 cm from the midline of each side of the depilated back of the rat.

**Wound contraction**—On 1, 3, 7, 10, and 14th day after wound creation, 6 animals in each group (group I to IV) were sacrificed randomly. The wound contraction was monitored by measuring the progressive changes in raw wound area,
planimetrically on a transparent paper. The tracing was then transferred to 1 mm graph sheet, from which the wound surface area was calculated. The evaluated surface area was then employed to calculate the percentage of wound contraction, taking the initial size of the wound as 100% by using the following formula:

\[
\text{Wound contraction } \% = (\text{original wound area} - \text{specific day wound area})/\text{original wound area} \times 100.
\]

**Tensile strength**—The tensile strength of a wound represents the degree of wound healing. Healing tissue along with normal skin at two ends was excised for tensile strength measurement using Tensile Testing Machine TKG-20 (Fine Testing Machines, Miraz, India). Strips of 8 mm width and 20 mm length were cut out from the excised tissue in treated and control animals and were loaded between the upper and lower holder of the machine in such a way that the effective load bearing size was 8 × 8 mm with the wound remaining in the centre. The total breaking load is measured in Newtons and the tensile strength was calculated by the following equation:

\[
\text{Tensile strength} = \frac{\text{total breaking load}}{\text{cross-sectional area}}.
\]

Meanwhile, the wound tissues of methanol treated group, Madecassol® group and control were excised to analyze histology, immunohistochemistry, reverse transcriptase polymerase chain reaction (RT-PCR) and Western-blot.

**Histopathology**—Samples at the end of the 14th day after treatments were fixed in 10% buffered formalin, processed and blocked with paraffin and then sectioned into 5 µm thickness and stained with hematoxylin and eosin (HE), Van Gieson’s (VG) and toluidine blue (TB) stains. Sections were analyzed and scored as poor (+), moderate (++) and good (+++) for epidermal or dermal re-modelling. Re-epithelization in epidermis, fibroblast proliferation, mononuclear and or polymorphonuclear cells, neo-vascularization and collagen depositions in dermis were analyzed to score the epidermal or dermal re-modeling. Van Gieson’s stained sections were checked for collagen deposition and toluidine blue stained sections checked for metachromatic staining of mast cells.

**Immunohistochemical assay of microvessel density (MVD) and vascular endothelial growth factor A (VEGFA) expression**—Wound tissue sections were deparaffinized and rehydrated followed by 3% H2O2 in methanol treatment for 10 min to block endogenous peroxidative activity. Then the sections were boiled in 0.01 mol/L citric acid for 20 min to retrieve the antigen. To block nonspecific binding, normal goat serum was applied for at 37 °C for 10 min, then the sections were reacted with mouse anti-rat CD31 monoclonal antibody (diluted 1:5, Abcam, UK) or mouse anti-rat VEGFA monoclonal antibody (diluted 1:5, Abcam, UK) at 37 °C for 1 h. Sections were washed with PBS and incubated with biotinylated goat antimouse antibody at 37 °C for 30 min and then horseradish peroxidase-labeled streptavidin at 37°C for 30 min. After 3, 3′-diaminobenzidine (DAB)/H2O2 staining and hematoxylin staining, sections were dehydrated, cleared, and mounted for observation. The images were analyzed by Image-pro-plus 6.0 software to calculate the area, density mean and integrated optical density (IOD) of positive expression. The average result of the five areas was recorded as the statistic data of this sample.

**Analysis of VEGFA gene expression by RT-PCR**—Total RNA was isolated from homogenized excised tissues using Trizol (Invitrogen, USA), and cDNA was synthesized using RT-PCR kit according to the manufacturer's protocol. The oligonucleotide sequences of the primers of VEGFA were 5'-TGCACC-CACGACAGGGA-3' for sense and 5'-TCACCCTTGGCTTGTCACAT-3' for antisense; while the sequences of primer of Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) used as a control were 5'-GGGCGTGAGCTGTCAGAAG-3' for sense and 5'-GCCACGATGCCAGGAA-3' for antisense. PCR conditions were standardized as follows: denaturation at 95 °C for 3 min, and then 30 cycles of denaturation for 20 sec at 94 °C, annealing for 30 sec at 55 °C and extension for 30 sec at 72 °C. The PCR products were separated using agarose gel, and photographed under UV. Band intensities were measured using Gel-Pro Analyzer 6.0 software (Media Cybernetics, USA) and were normalized to those for GAPDH.

**Analysis of VEGFA protein expression by Western-blot**—Excised tissues were homogenized with lysis buffer and centrifuged. The supernatant lysate were mixed with 2 x sodium dodecyl sulfate (SDS) sample buffer (0.5 M Tris-HCl pH 6.8, 10% SDS, 5% glycerol and 5% β-mercaptoethanol). Equal amounts
of denatured proteins were resolved by 10% SDS-PAGE and then transferred to nitrocellulose membranes. After blocking for 1 h with phosphate-buffered saline containing 0.1% Tween 20 and 5% powdered skim milk at room temperature, the membranes were incubated with mouse anti-rat VEGFA monoclonal antibody (diluted 1:1000, Abcam) 37 °C for 4 h in 5% powdered skim milk buffer, washed thrice with phosphate-buffered saline with 0.1% Tween 20 and then incubated with secondary antibody anti-mouse HRP (diluted 1:5000). GAPDH antibody (diluted 1:500) was used as a control. All bands were detected using ECL Western-blot kit. Protein band intensities were determined using Gel-Pro Analyzer 6.0 software and were normalized to those for GAPDH.

**Statistical analysis**—Every 6 of 30 animals per group were sacrificed randomly at 5 different time points. All the presented data was expressed as mean ± SD, and the statistical significances among three groups were analyzed by one-way ANOVA, and then differences among means were analyzed with Fisher’s least significant difference t-test using the SPSS version 13.0 software (SPSS Inc., USA). P < 0.05 was considered as statistically significant.

**Results**

The preliminary phytochemical screening of *P. beddomei* thallus extracts showed presence of flavonoids, amino acids, amino sugar, amines, tannins, phenolic substances and proteins respectively in methanolic and aqueous extracts.

Presence of sterols and triterpenoids was detected visually by spraying with 5% H₂SO₄ in methanol (Fig. 1a), Tannins showed color reaction by ferric chloride solution (Fig. 1b) whereas flavonoids were confirmed by yellow fluorescence on spraying with aluminium trichloride solution (Fig. 1c). Amino acids were confirmed purple color on spraying with ninhydrin solution (Fig. 1d).

Significant promotion of wound healing was induced by the methanolic and aqueous extracts and is comparable to the reference drug. In the excision wound model, the methanolic extract treated groups of animals showed 56.5 ± 0.7% contraction on the wounds at 7th day. The same extract demonstrated 80.6 ± 0.18% contraction on the 14th day which was close to contraction value of the reference drug Madecassol®. Aqueous extract showed 46.2 ± 0.5% (7th day) and 74.3 ± 0.16% (14th day). However, no significant difference among the treated groups was noticed in the initial periods (Table 1).

Tensile strength of the wounds in animals treated with the methanolic extract demonstrated the highest value followed by reference drug and aqueous extract at day 10. Topical application of the methanolic and

<table>
<thead>
<tr>
<th></th>
<th>Wound contraction</th>
<th>Tensile strength</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1  3  7  10  14</td>
<td>1  3  7  10  14</td>
</tr>
<tr>
<td>C</td>
<td>4.5±0.01 9.2±0.2 26.3±0.04 39.2±0.04 3.4±0.01 5.6±0.02 7.6±0.03 8.4±0.01 10.2±0.1</td>
<td></td>
</tr>
<tr>
<td>ME</td>
<td>5.2±0.03 13.8±0.1 56.5±0.7 61±0.09 80.6±0.18 8.2±0.03 10±0.03 12.8±0.02 16.5±0.01 21.5±0.01</td>
<td></td>
</tr>
<tr>
<td>AE</td>
<td>4.8±0.02 12.4±0.02 46.2±0.05 54.6±0.07 74.3±0.16 7.6±0.02 9.5±0.01 11.5±0.1 13.2±0.01 18.5±0.01</td>
<td></td>
</tr>
<tr>
<td>M®</td>
<td>3.5±0.03 14.2±0.01 50.2±0.04 63.5±0.05 85.6±0.2 9.8±0.03 11.2±0.02 13.1±0.2 17.2±0.02 20.1±0.01</td>
<td></td>
</tr>
</tbody>
</table>

*Table 1—Effect of P. beddomei* methanolic (ME), aqueous (AE) extracts and Madecassol (M®) on wound contraction (%) and tensile strength (N/cm²) in rats. [Values are mean ± SD]
aqueous extract on the incision wound model demonstrated a remarkable improvement in wound tensile strength compared to other groups (Table 1).

**Histopathological examination**—Histopathological studies showed significant neovascularization, epithelialization and fibroblast in methanolic treated groups. In a similar way, group treated with Madecassol® showed matured epidermis with keratinization and mature hair follicles, fibroblasts in dermis that are the proof of completion of healing (Table 2). The reference drug and the control groups demonstrated delayed wound healing processes compared to methanol treated groups. In comparison with the control and the reference drug groups, faster re-modeling were noticed in methanolic extract treated groups. The best re-modeling, particularly, re-epithelization were detected with the methanol extract group (Fig. 2 a-i). On the other hand, faster

<table>
<thead>
<tr>
<th>Groups</th>
<th>Wound healing processes</th>
<th>Healing phases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AE S U RE FP CD MNC PMN</td>
<td></td>
</tr>
<tr>
<td>ME</td>
<td>+ - ++ ++ ++ - -</td>
<td>+ +++ + - /+ ++</td>
</tr>
<tr>
<td>M®</td>
<td>+ - +++ + ++ - -</td>
<td>+ /+ ++ -/+ ++</td>
</tr>
<tr>
<td></td>
<td>+/++ - ++ ++ +++ -</td>
<td>++ /++ ++ +</td>
</tr>
</tbody>
</table>

S: Scab; U: Ulcus; RE: re-epithelization; FP: fibroblast proliferation; CD: collagen depositions; MNC: mononuclear cells; PMN: polymorphonuclear cells; NV: neovascularization; MC: Mast cells; I: inflammation phase; P: proliferation phase; R: re-modeling phase.

HE, VG and TB stained sections were scored as poor (+), moderate (++) and good (+++) for epidermal and/or dermal re-modeling.

Fig. 2—Histopathological view of wound healing and epidermal/dermal re-modeling in the P. beddomei extracts and Madecassol® administered animals. Skin sections show the hematoxylin and eosin (HE) stained epidermis and dermis in a, and the dermis stained with Van Gieson’s (VG) and toluidine blue (TB) in b and c respectively. The original magnification was 40× and the scale bars represent 25 µm for figures in a, d, g, and the original magnification was 400× and the scale bars represent 100 µm for both b, c, e, f, h and i. Data are representative of six animal per group. (2 a, b, c) 14 days old wound tissue treated with Madecassol®; 14 days old wound tissue treated with methanol extract (2 d, e, f) and 14 days old wound tissue treated as control group (2 g,h,i).
keratinization characterized with minor intraepithelial cornification was seen in methanolic extract group. Weak foreign body reaction, superfluous process in wound healing, characterized with a few foreign body giant cells, which generally localized in peripheral sides of some hair follicles were detected in all groups except for the control group.

Immunohistochemical analysis of MVD and VEGFA expression—MVD, area and IOD of VEGFA expression after the 3rd day were at the climax among all the groups coupled with healing process (Fig. 3 a-f). The above mentioned parameters of *P. beddomei* methanol extract treated group and Madecassol® group were higher than that of control group (*P < 0.05*). Additionally, compared with Madecassol® group, the MVD and VEGFA expression of *P. beddomei* methanol extract treated group were higher (*P < 0.05*).

**VEGFA gene expression by RT-PCR and VEGFA protein expression by Western-blot**—VEGFA gene expression in wounds determined on 3rd day by RT-PCR was seen in all groups (Fig. 4). Expression of *P. beddomei* methanol extract group and Madecassol® group was higher comparatively than that of control group (*P < 0.05*). Furthermore, the expression of *P. beddomei* methanol extract group was higher than that of Madecassol® group (*P < 0.05*). Similarly, the VEGFA protein expression in wounds was up-regulated in both *P. beddomei* methanol extract groups and of Madecassol® group as compared with control (*P < 0.05*) (Fig. 5). Moreover, the VEGFA protein was expressed more in *P. beddomei* methanol extract group than Madecassol® control (*P < 0.05*). The VEGFA gene or protein expression showed no significant difference among the three groups at other time points (Table 3).

**Discussion**

More than 80% of the world population still depends upon traditional medicines for their ailments, especially for wound management. Phytomedicines induce healing and regeneration of the tissue by multiple mechanisms. The present results show that methanolic and aqueous extracts...
enhanced wound contraction and tensile strength; this is in agreement with histopathological findings which showed a faster remodeling particularly, re-epithelization, keratinization with minor intraepithelial cornification. In incision wound, the increase in tensile strength of treated wounds may be due to the increase in collagen concentration and stabilization of the fibers\textsuperscript{18,19}. In excision wound, both the extracts showed faster healing compared with control group and more remarkably with methanolic extract as compared with aqueous extract. The faster wound contraction rate of the methanolic extract may be due to stimulation of interleukin-8, an inflammatory $\alpha$-chemokine which affects the function and recruitment of various inflammatory cells, fibroblasts and keratinocytes. It may increase the gap junctional intracellular communication in cultured fibroblasts and induces a more rapid maturation of granulation tissue\textsuperscript{19}. Histological observation suggested that the phytochemical content of the methanol extract may be responsible for collagen formation at the proliferative state, which is contributed by increased fibroblasts content\textsuperscript{19} (Fig. 2).

Similarly, the methanolic extract of \textit{P. beddomei} could up-regulate immunohistochemical, transcriptional and translational level of VEGFA expression and increase the amount of new-formed capillary at inflammatory phase coupled with the percent wound contraction at granulation formation and scar remodeling phase. The enhanced effect of the extracts on angiogenesis and wound healing did not happen simultaneously. Prior to promoting wound healing, the methanolic extract demonstrated a stronger angiogenic effect, than Madecassol\textsuperscript{®} treated. So we can infer that the methanolic extracts of \textit{P. beddomei} promote wound healing effectively characterized by reduced healing time, probably due to their potential angiogenic property. Since neovascularity, an indicator of immature granulation tissue, diminishes gradually as wound mature\textsuperscript{16}, the extracts did not influence MVD and VEGFA expression at late stage of healing process resulting in an acceleration of wound maturity. In addition, after the extracts were applied, the rats did not show any physiological symptom of restlessness or scratching wound site, probably suggesting that the extracts did not cause irritation or pain to the rats. However, whether the extracts cause other side effects needs further formal and systemic investigations.

Table 3—Relative expression of VEGFA gene and protein in rats showed the highest with methanolic group and lowest with control group (* compared with control group; **compared with Madecassol\textsuperscript{®} group).

<table>
<thead>
<tr>
<th></th>
<th>Methanolic extract group</th>
<th>Control</th>
<th>Madecassol\textsuperscript{®} group</th>
</tr>
</thead>
<tbody>
<tr>
<td>gene</td>
<td>0.74**</td>
<td>0.18</td>
<td>0.44*</td>
</tr>
<tr>
<td>Protein</td>
<td>1.03**</td>
<td>0.16</td>
<td>0.22*</td>
</tr>
</tbody>
</table>

Phytochemicals in \textit{P. beddomei} extracts such as sterols, phenols, triterpenoids, tannins and flavonoids synergistically affect the function of vessels in several aspects, involved in newly-formed micro-vessels formation and VEGFA expression in the wound healing process\textsuperscript{16}. Fatty acid extracts of \textit{Lucilia sericata} larvae promote cutaneous wound healing by angiogenic activity\textsuperscript{15,21}. Secondary metabolites are mediators of several events such as cellular growth, angiogenesis and extracellular matrix synthesis\textsuperscript{23}. It is reported that polyphenols can regulate epithelial cell proliferation \textit{in vitro}\textsuperscript{23} and angiogenesis \textit{in vivo} by ERK, p38 MAPK or PI3K signal pathway\textsuperscript{24}.

It is well known that the presence of polyphenols possess an anti-oxidative effect related to reacting with reactive oxygen species (ROS) called peroxidation. At the early stage of healing process, inflammatory cells such as neutrophils and macrophages release a high amount of ROS by an oxygen consuming respiratory burst\textsuperscript{25}. The ROS play...
a dual regenerative role for wound metabolism. It is reported that low concentration of oxygen free radicals in the wound site can promote angiogenesis by inducing VEGFA expression in keratinocytes and macrophages as well as stimulate collagen production. However at high concentration, oxygen free radicals exhibit obvious tissue damage action and induce apoptosis of wound repair cells. Better collagenation, indirectly seen under the influence of this plant extract, may be because of the presence of phytochemicals, which is responsible for the free radical scavenging activity and is believed to be one of the most important components of wound healing. In addition, the decrease of apoptosis of endothelial cells induced by ROS may also contribute to the promotion of angiogenesis. To sum up, the angiogenic activity of *P. beddomei* extracts is related to its synergistic effects of both proliferation and anti-oxidation on endothelial cells.

In conclusion, the present study demonstrate that the thallus of *P. beddomei* promote wound healing activity in rat at preclinical study. The methanolic extract showed remarkable wound healing activity and further studies are warranted with purified constituents to comprehend the complete mechanism of wound healing activity of *P. beddomei*.

### References