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Conclusions The present study showed that only polar constituents prevent mast-cell degranulation whereas non-polar constituents were unable to prevent mast-cell degranulation.

75
Investigations on wound-healing activity of leaves of *Ocimum basilicum* L.

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Objectives Indian basil or *Ocimum basilicum* L. is a holy plant and widely used to cure various ailments including skin diseases. In the present investigations, scientific validation of the wound-healing properties of leaves of *O. basilicum* was carried out along with investigation into the quality parameters of the extracts used for the study.

Methods Fresh leaves of *Ocimum basilicum* L. were collected, dried, powdered and extracted with three different solvents (water, alcohol and hydro-alcohol (25%)) by maceration for 72 hours with occasional shaking. All the extracts were concentrated under reduced pressure and at lower temperature using a rotary evaporator; after this they were dried. High-performance thin-layer chromatography fingerprinting of the dried extracts was developed. Swiss albino mice of 25–30 g in weight were used for the experiment and approved by the ethical committee of our institute. Animals were anaesthetized under ketamine anaesthesia (intraperitoneal 10 mg/kg) and an area of approximately 4 cm^2 on the dorsal surface was clipped free of hair and swabbed with 70% ethanol. The cleared dorsal surface of the skin was marked with a sterile circular (10 mm diameter) stainless steel stencil. A full-thickness wound was created by excising the skin flap in an aseptic environment using sterile scissors and forceps. The mice were divided into five groups (n = 5). Group I was kept as a control group, group II was kept as a positive control and treated with marketed formulation (0.5% w/w povidone iodine ointment) and groups III, IV and V were treated with the aqueous, hydro-alcoholic and alcoholic *O. basilicum* extracts topically on the wound daily up to the 16th day after wounding.

Results To evaluate the wound-healing potential of *O. basilicum*, hydroxyproline content and percentage wound contraction were estimated. Hydroxyproline content is an index of collagen and measures the synthesis of neocollagen, which is an indicator of improved condition of wound healing in animals. Hydroxyproline was estimated (Woessner 1961) from granulation tissues of animals on the 16th day after wound creation. The levels of hydroxyproline were found to be 6.40 ± 1.09, 7.10 ± 2.53, 10.11 ± 1.26, 14.95 ± 1.87 and 8.52 ± 1.28 mg/g of tissue in groups I–V, respectively. Hydroxyproline level was significantly higher ($P < 0.05$) in animals treated with hydro-alcoholic extract (group IV). Wound contraction indicates the rate of reduction of an unhealed area during the course of treatment; the greater the reduction the better the efficacy of the medication (Peacock 1984). Wound contraction was measured by tracing the wound margins at 4-day intervals on transparent graph paper with a millimetre scale to an accuracy of 0.05 mm. The wound-contraction data are shown in Table 1.

Conclusions In conclusion, the hydro-alcoholic extract of *O. basilicum* leaves showed significant wound-healing properties in this excision-wound model.

Table 1 Percentage of wound healing in mice from groups I–V

Group	Wound healing (%)			
	Day 4	Day 8	Day 12	Day 16
I (control)	43.68 ± 4.55	55.46 ± 2.01	72.52 ± 1.42	79.90 ± 0.82
II (positive control)	45.35 ± 4.53	60.76 ± 1.01	75.14 ± 1.46	82.70 ± 0.66
III (aqueous extract)	43.24 ± 3.42	69.36 ± 2.44	78.10 ± 0.70	86.52 ± 0.71
IV (hydro-alcoholic extract)	43.38 ± 1.86	74.58 ± 4.25	95.55 ± 0.85	98.42 ± 0.15
V (alcoholic extract)	40.72 ± 1.90	65.42 ± 4.19	76.78 ± 1.48	83.36 ± 1.04

Peacock, E. E. (1984) In *Wound repair*, 3rd edn, Peacock, E. E., ed. Saunders, pp 39–55

Woessner, J. J. F. (1961) *Arch. Biochem. Biophys.* **93**: 440–447

SESSION 2
Analytical Chemistry

76
High-performance liquid chromatography method for simultaneous determination of gallic acid and ellagic acid in herbal extract and formulations

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Objectives This study was performed for the development and validation of reverse-phase high-performance liquid chromatography (RP-HPLC) methods for standardization of two widely used herbs, the whole plant of *Phyllanthus amarus* L. and seed extract of *Syzygium cumini* L. Two marker compounds, gallic acid and ellagic acid, were quantified from the methanol extracts as well as from commercial formulations using RP-HPLC. This paper presents an isocratic elution system for the separation of gallic acid and ellagic acid. Method validation was performed as recommended in the International Conference on Harmonisation (ICH) guidelines.

Methods Reference standards of gallic acid and ellagic acid (purity 98%, w/w) were from Natural Remedies (India) and all chemicals used were of analytical grade. Methanol extracts were prepared from the whole plant of *P. amarus* and seed of *S. cumini* and their formulations from three different places. A Thermo ODS Hypersil C_{18} (250 mm × 4.6 mm, 5 μm) column was used. Separation was achieved using a mobile phase consisting of 5 mM potassium dihydrogen phosphate (pH 3.2)/acetonitrile (41:9) at a flow rate of 1.0 mL/minute. The eluent was monitored using UV detection at a wavelength of 280 nm. Calibration curves were prepared using standard solution in a range of 0.8–8 μg/mL for gallic acid and 2–20 μg/mL for ellagic acid. The validation parameters addressed were specificity, precision, accuracy, linearity, limits of detection and quantification and the stability of gallic acid and ellagic acid in the mobile phase.

Results HPLC method development and optimization were done after tracking several elution systems. The study revealed that the gallic acid and ellagic acid were well resolved from *P. amarus* and *S. cumini* extracts as well as their formulations, with retention times of 3.8 and 10.3 minutes respectively. Good linearity was achieved in the investigated ranges for both analytes. Correlation coefficients were $R^2 = 0.998$ for gallic acid and $R^2 = 0.999$ for ellagic acid. The values for limit of detection (LOD) were 0.2 and 1 and limit of quantification (LOQ) values were 0.8 and 1.6 respectively. The relative SD (RSD) values for injection repeatability, analysis repeatability and for the intra-assay and inter-assay precision were lower than 2.0% of the peak area. The percentage recovery values at three different levels of gallic acid were 101.33, 99.04 and 101.14% and for ellagic acid were 97.20, 101.38 and 99.00%. The gallic acid and ellagic acid contents found were 0.41 ± 0.03 and 0.13 ± 0.02% respectively in *P. amarus* and 1.15 ± 0.02 and 0.36 ± 0.01% in *S. cumini* respectively.

Conclusions The developed HPLC method is precise, specific and accurate for determination of both the marker compounds. In previously reported work the time required for each run was high. But the method that we have developed proves that repeatable and selective analysis of these two active components in herbal drugs as well as in pharmaceutical formulations requires less time. It can also be used in routine quality control of herbal raw materials as well as formulations containing either or both of these compounds.

77
The effect of complex coordination on gallium determination using ion chromatography

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Objectives Interest in the therapeutic use of gallium is increasing due to the discovery of its effectiveness as an anti-tumour and anti-microbial agent (Bernstein 1998). However, gallium strongly coordinates with many organic and inorganic ligands and these complexes can display very different physico-chemical properties (Houghton 1979). The gallium species present may also influence the accuracy of the assay used to quantify it. To enable the design of dosage form gallium must be accurately quantified irrespective of the coordination species present. The aim of this study was to develop a simple, rapid method for determining gallium content in a range of coordination complexes in a simple vehicle and a biological matrix.