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Validated method for quality assessment of henna (*Lawsonia inermis* L.) leaves after postharvest blanching and its cosmetic application

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ABSTRACT

Henna (*Lawsonia inermis* L., Family- Lythraceae) has been used for number of cosmetic purposes, including body painting, palm colouring and dyeing of hair. In spite of huge demand for cosmetic applications, no validated analytical method is available for the quality assessment of henna. Therefore, present validated method demonstrates the simultaneous quantification of eight marker compounds. Chemical markers, namely **1**) gallic acid, **2**) quercetin, **3**) fraxetin, **4**) luteolin-7-*O*- glucoside, **5**) *p*-coumaric acid, **6**) lawsone, **7**) luteolin and **8**) apigenin were chosen for the quality assessment of henna. The method was also applied to assess the effects of postharvest treatments (PHT) on the quality of henna leaves as well its derived product i.e. oil mixed paste. Both the chemical and thermal blanching treatments severely ($p < 0.5$) changed the content of targeted secondary metabolites (**1**–**8**). The contents of fraxetin and lawsone were found to increase on deep freezing, remaining six phytochemicals reduced significantly on blanching. Luteolin-7-*O*- glucoside in henna was most susceptible to both salt and liquid nitrogen treatment. Additionally, the effects of mixing of oils (olive, castor oil, and mentha) on modulation of chemical markers and color intensity over palm were also evaluated. We observed a significant increase in the color intensity attributed to mixing of castor » menthol > olive oil. The apigenin content was about 2.4 times higher in olive oil mixed henna paste than control, while, fraxetin content reduced to half. Mixing of castor oil in henna paste has produced the most intense color; while the mentha oil facilitated the persistence action when applied for palm ornamentation.

1. Introduction

Henna (*Lawsonia inermis* L., Family- Lythraceae), is a native of North Africa and also cultivated in India, Middle East, Arabian Peninsula and other Asian countries on a commercial scale. In India, it is popularly known as *Mehndi* and used during various socio-cultural activities such as marriage, festivals, etc. (Saksena, 1979). It is a branched glabrous shrub-like tree and sustains for 20–25 years under the coppice system (Shukla et al., 2012). The popular use of henna comprises ornamentation of palm, soles, feet, nails, dying of hairs, beard, and eyebrows (Semwal et al., 2014; Chand and Jangid, 2007). It is one of the secondary agriculture oriented crops of India. The Government of India has promoted the secondary agriculture of prioritized medicinal and aromatic plants, in order to meet the domestic and global requirement. However, henna has not been put under such category due to the reason its steadily incorporation and stabilization into the main agricultural systems (Ved and Goraya, 2007). Being the major export crop cultivated in one of the rainfed region of India i.e. Rajasthan state, henna shares 90% of the total production of the country (Chand and Jangid, 2007). Unlike other botanical, the major part of the henna production is exported to African countries, Europe, Middle East and the USA.

Globally, henna is used in various traditional and folklore system of medicine for the treatment of a wide range of seemingly unrelated ailments such as, dysentery, gonorrhoea, bronchitis, wound healing, boils, conjunctivitis, pimples, dandruff, scabies, jaundice and other skin disorders e.g. itching dysuria, bleeding disorder, prurigo etc. (Agarwal et al., 2014; Akram et al., 2014; Semwal et al., 2014; Singh et al., 2015).

Recent research studies have also shown that henna possesses a wide range of biological activities viz. anti-fungal, anti-bacterial, anti-parasitic, virucidal, analgesic, hepatoprotective, anti-inflammatory, immunomodulatory, anthelmintic, anti-cancer, anti-oxidant, and allelopathic (Ahmadian and Fakhree, 2009; Dhaouadi et al., 2015; Imam et al., 2013; Jeyathilakan et al., 2012; Li et al., 2014; Nesa et al., 2014; Sharma et al., 2009; Singh

Abbreviations: LI, *Lawsonia inermis*; RP-HPLC, reverse phase liquid chromatography; UAE, ultrasonic assisted extraction; PHT, postharvest treatments; PPD, p-phenylenediamine.

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et al., 2015). The medicinal value of henna is attributed to its naturally occurring secondary metabolites. However, the adulteration of *p*-phenylenediamine (PPD), a potent contact allergen, has also been reported in the so-called temporary black henna tattoo (Almeida et al., 2012).

The phytochemical investigations of *L. inermis* has revealed more than a hundred secondary metabolites of diverse chemical nature predominantly coumarins, flavonoids, naphthoquinones, quinoids, naphthalene derivatives, triterpenoids, coumarins, organic acids, xanthones, phenolics, and phenolic glycosides (Ahmed et al., 2000; Liou et al., 2013; Mengoni et al., 2016; Semwal et al., 2014; Singh et al., 2015). The presence of diversified biological active chemical armory in henna treats a range of veterinary and human health ailments. Its capability to grow under stress conditions of the semi-arid environment has most probably geared the enormous biosynthetic pathways towards the production of such diverse secondary metabolites.

Several postharvest approaches viz. cooling, thermal, and chemical (calcium chloride, sodium carbonate and bi-carbonate) are adopted for extending shelf life and maintaining the quality of the farm produce. Calcium chloride treatment is an economical processing step that minimizes damage in plant tissue. Post-harvest calcium dips reduce respiration; facilitate cell wall integrity, tissue firmness, and delays membrane lipid catabolism.

To the best of our knowledge, no study has been reported so far on postharvest treatment effect on *L. inermis*. Thus, the aim of this investigation was to develop a simple, and efficient chromatographic technique for simultaneous quantification of eight chemical markers viz. **1**) gallic acid, **2**) quercetin, **3**) fraxetin, **4**) luteolin-7-*O*- glucoside, **5**) *p*-coumaric acid, **6**) lawsone, **7**) luteolin and **8**) apigenin in dried henna powder and postharvest treated (PHT) samples. Additionally, the effect of three oils viz. olive, castor, and mentha were evaluated for phytochemical changes on blending with henna paste preparation and their effect on the ornamentation capacity to palm (intensity and persistence).

2. Materials and methods

2.1. Plant material and sample preparation

The whole plant of *Lawsonia inermis* (LI) was *collected* from the research farm of the Central Institute of medicinal and aromatic plants (Voucher specimen number 14555). The plant material was stored at room temperature following good storage practices (WHO, 2009). The shade dried and milled material was extracted by different techniques and organic solvents such as hexane, chloroform, ethylacetate, methanol, and water. The extraction efficiency of different solvents of henna markers was optimized with various operating parameters. The optimum extraction was achieved with ultrasonic assisted extraction (UAE) techniques using methanol solvents. The UAE method was used for sample preparation in further experiments of validation and post-harvest quality assessment. The respective extracts were pooled, concentrated under vacuum using a rotary evaporator (Buchi, Switzerland), reconstituted with 1.0 mL of the organic component of the mobile phase (methanol/aceonitrile-1:1), centrifuged at 7280 *g* for 5 min. Prior to HPLC analysis, samples were filtered with nylon 0.45 μm filter paper.

2.2. Chemicals and standard

All the eight reference standards, namely- **1**) gallic acid, **2**) quercetin, **3**) fraxetin, **4**) luteolin-7-*O*- glucoside, **5**) *p*-coumaric acid, **6**) lawsone, **7**) luteolin, and **8**) apigenin were purchased from Sigma-Aldrich, USA. HPLC grade acetonitrile, methanol, and water were procured from E. Merck, Mumbai, India. All the solvents and sample solutions were filtered through 0.45 μm Millipore membrane filters on filtration unit (Millipore, Billerica, MA, USA) before use.

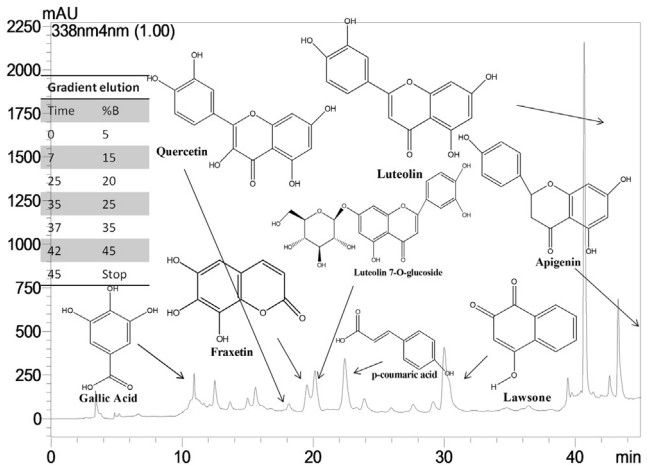

Fig. 1. Reference standards of *L. inermis* and their elution sequence under experimental chromatographic conditions.

2.3. Apparatus

The chromatographic system from Shimadzu (Shimadzu Corporation, Kyoto, Japan) was composed of LC-20AD pumps, Rheodyne manual injector, CTO-20A oven, and SDP M20 diode array detector. Chromatographic data acquisition and processing were performed using LC–MS Solution 3.21 software (Shimadzu). A model CBM-20 Shimadzu interface was used to send the signals from the detector to the computer.

The chromatographic separation was achieved by a gradient elution using a Phenomenex C_{18} (4.6 × 250 mm, 5 μm) at 35 °C. The mobile phase comprised the acetic acid (0.5%, v/v) in water (solvent A) and acetonitrile-methanol (50:50%, v/v) (solvent B). The gradient elution started with 5% B with a flow rate of 1.0 mL/min (Fig. 1). Prior to use, the mobile phase was degassed for 15 min by ultrasonication (Microclean-109, Oscar Ultrasonics, Mumbai, India 30.0 × 25.0 × 12.5 cm, 34 ± 3 kHz, PZT Sandwich type six transducer, 250W). Samples and the mobile phase were filtered through a 0.45 μm nylon membrane using solvent filtration apparatus (Millipore, USA). Detector wavelength was set at 338 nm and the injection volume was 20 μL. The data acquisition was performed in the range of 200–400 nm to monitor any possible co-elution in plant samples, Chromatogram at 338 nm wavelength was selected for simultaneous quantitation on the basis of the optimum chromatographic signal response of all eight targeted phytochemicals of henna.

2.4. Standards solutions

The stock solutions of all eight reference standards were prepared in methanol at 5.0 mg/ mL and stored at 4 °C. The dilution was done for each experiment. Five concentrations (m = 5) for each reference were used. Each concentration was analyzed 3 times ($n = 3$) for 3 days ($k = 3$). The extract solution of *L. inermis* was diluted with the organic component of the mobile phase for the preparation of the validation standards and spiking with three known concentrations of a stock mixture of the reference standards. Each validation standard too was analyzed in triplicate i.e. ($n = 3$) for 3 days ($k = 3$).