DETERMINATION OF LAWSONE CONTENT IN FRESH AND DRIED LEAVES OF LAWSONIA INERMIS LINN. AND ITS QUANTITATIVE ANALYSIS BY HPTLC

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ABSTRACT

The present study was carried out to determine the content of lawsone in fresh and dried leaves of Lawsonia Linn. The measurement containing high lawsone content was proceeded for further investigation by making hydro-alcoholic extract of the chosen plant part and its phytochemical analysis was also performed. The test extract was further screened for quantitative estimation of lawsone content using high performance thin layer chromatography. The chromatograms were developed using toluene: ethyl acetate: formic acid (5:4:0.5) as solvent system. Dried leaves of Lawsonia had 5.3 mcg/ml lawsone content while in case of dried leaves, 6.9 mcg/ml lawsone content was observed. The chromatogram of hydroalcoholic extract of leaves of Lawsonia Linn showed the presence of 4.56 mcg/ml lawsone.

KEYWORDS: Chromatography, extract, hydroalcoholic, lawsone, Lawsonia Linn., quantitative analysis.

INTRODUCTION

Lawsonia inermis Linn (Lythraceae), most commonly known as ‘henna’ invites attention of the investigators worldwide for its pharmacological profile ranging from anti-inflammatory to anti-cancer activities.1 L. inermis is a much branched shrub that grows mainly in middle east of Africa.2 The leaves of L. inermis having the highest yield of lawsone are confined to India, mainly in Punjab and Gujrat and to a small extent in Rajasthan and Madhya Pradesh.3 The principal colouring substance of henna is a red-orange colored molecule (lawsone, 2-hydroxy-1, 4 naphthaquinone) having molecular formula, C16H8O3 and melting point of 190°C, present in dried leaves in a concentration of 1-1.4% w/w.4,5,6 Lawsone is chiefly responsible for the colorant property of henna leaves.3 Lawsone is available as yellow to mustard coloured powder which is practically insoluble in water at 0.2%, soluble in 95% ethanol at 0.5%, soluble in methanol at 1%. Lawsone is proposed to be used as a non-oxidising hair colouring agent at a maximum concentration of 1.5% (typical concentration 1.26%) in the finished cosmetic product.7 The process which is mainly used for the extraction of lawsone from leaves of henna has been patented. Upadhyay et al in 2010, confirmed that the quantitative estimation of leaves of Lawsonia collected in different seasons showed variations in the active ingredient (lawsone). The leaves obtained in march season contain less lawsone content in comparison to october – november season leaves of Lawsonia.8

Henna is not a sacred plant as such, but it is supposed to symbolize prosperity, fertility and happiness. It is widely used in a variety of religious and social ceremonies in India.9 The leaves of Lawsonia have bitter bad taste and used in vulnerary, diuretic, headache, hemicranias, lumbago, bronchitis, boils, ophthalmia, syphilis, sores, amenorrhoea, scabies and spleen diseases and also favours hair growth.2 In the present study, the content of lawsone in fresh and dried leaves of Lawsonia was detected and the measurement containing high content of lawsone was proceeded for further investigation by making hydro-alcoholic extract of the chosen plant part since hydroalcoholic extract is known to haul out a variety of chemical constituents (both polar and non-polar).

Therefore, it was considered that most of the active components including lawsone has been extracted out in the hydroalcoholic extract. The test extract was further screened for quantitative estimation of lawsone content using high performance thin layer chromatography.

MATERIALS AND METHODS

Synthetic lawsone was purchased from Yucca enterprises, Mumbai, India. All the chemicals used were of analytical grade.

Collection and authentication of plant material

Fresh leaves of Lawsonia were collected from botanical garden of Maharshi Dayanand University, Rohtak and authenticated by Dr. H. B. Singh, Scientist F & Head, Raw Materials Herbarium & Museum, National Institute of Science Communication and Information Resources (NISCAIR) New Delhi, India (voucher specimen no: NISCAIR/RHMD/Consult/-2011-12/1990/290).

Figure 1. Fresh leaves of L. Inermis
Quantitative estimation of lawsone in fresh and dried leaves of *L. inermis*
Quantitative estimation of lawsone in fresh and dried leaves was carried out using spectrophotometric method in which 100 mg powdered leaves of *L. inermis* were soaked in 10 ml methanol for 2 h. Thereafter, the mixture was centrifuged at 5000 rpm for 20 min and the clear supernatant was separated out into a test tube. The absorbance of supernatant was read out at 452 nm. A calibration curve was prepared by plotting the concentration versus absorbance at the same wavelength in the concentration range 10-200 µg/ml of pure lawsone. The lawsone content in fresh and dried leaves was then calculated in µg/ml. All the observations were taken in triplicate.

**Processing and preparation of hydro alcoholic extract of dried *L. inermis* leaves**
The leaves of *L. inermis* were shade dried as specified in WHO and dried leaves were ground using pestle and mortar and carefully packed in sterile polythene bags, weighed and stored under room temperature until used. The powdered material was extracted with 50% ethanol using soxhlet apparatus. After the extraction was complete, solvent was evaporated under reduced pressure in a rotary vacuum evaporator to give a dark brown viscous mass.

Quantification of lawsone in hydroalcoholic extract of *L. inermis* using high performance thin layer chromatography (HPTLC)
The hydro alcoholic extract of *L. inermis* was subjected to HPTLC analysis using HPTLC LINOMET 5 applicator and CAMAG 3 scanner with the aid of wincat software for characterization. The chromatograms were developed using solvent system v.i.z. toluene: ethyl acetate: formic acid (5.5: 4.0: 0.5).\(^{10,11}\)

**Preparation of standard and sample**
For standard solutions, 10mg of pure lawsone was dissolved in 10ml methanol. The solution was diluted in a concentration range of 10-200 mcg/ml. The sample solution of hydro alcoholic extract of *L. inermis* was prepared at a concentration of 10 mg/ml.

**RESULTS AND DISCUSSION**
As per the quantitative analysis of lawsone in fresh and dried leaves of *L. inermis*, it was found that dried leaves contained more lawsone as compared to fresh leaves. The absorbance of fresh and dried leaves was found to be 0.549 and 0.688 respectively. From the standard plot of lawsone (Figure 3), it was determined that dried leaves of *L. inermis* had more lawsone as compared to the fresh leaves.

The naphthaquinone fraction of hydroalcoholic extract of the leaves of *L. inermis* was characterized using HPTLC. The chromatograms were developed using toluene: ethyl acetate: formic acid (5.5: 4.0: 0.5) as solvent system at 254 nm and 366 nm and were compared with the chromatograms developed by pure lawsone. The chromatogram of hydroalcoholic extract of leaves of *L. inermis* showed the presence of 4.56 mcg/ml lawsone.
Lawsone, the main component of *L. inermis*, is not only a colouring agent but also possess diverse assortment of activities. In the present study, quantitative analysis of lawsone was carried out using fresh and dried leaves of *L. inermis*. Before going for the extraction of leaves of *L. inermis*, there was bewilderment whether to use fresh or dried leaves. To some extent, this perplexity can be cleared with the help of spectrophotometric analysis. The value for the concentration of lawsone (in mcg/ml) in fresh and dried leaves was calculated and found to be 5.3 mcg/ml and 6.9 mcg/ml for fresh and dried leaves respectively. On the basis of these findings, the dried leaves had more lawsone content as compared to fresh leaves. Further we also prepared the hydro alcoholic extract of leaves of *L. inermis* and phytochemical investigation of prepared extract showed the presence of carbohydrates, gums and mucilage, tannins and phenolic compounds, flavonoids, steroids and sterols. The hydroalcoholic extract of dried leaves of *L. inermis* was screened for quantitative analysis using HPTLC and its content was found to be 4.56 mcg/ml.
CONCLUSION
Henna finds applications in the treatment of a variety of disorders like vulnerary, diuretic, headache, hemicranias, lumbago, bronchitis, boils, ophthalmia, syphilitis, sores, amenorrhoea, scabies and spleen diseases. The key role behind its therapeutic efficacy is being played by its active constituent, lawsone, which can be further explored by incorporating this compound into novel dosage forms. The studies are further going on to make lawsone an efficient and potent drug molecule by complexing it with suitable legands. Therefore, efforts must be put forward to identify novel, natural and safe legands that may positively interact and synergistically affect the therapeutic efficacy of lawsone.

REFERENCES
7. Opinion of the scientific committee on cosmetic products and non-food products intended for consumers concerning lawsone COLIPA n° C146 adopted by the SCCNFP during the 21st plenary meeting of 17 September 2002 SCCNFP/0583/02, final evaluation and opinion on: lawsone.

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