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Effect of Solvents on Phytochemical Extraction and Effect of Packaging on Lawsone Content in *Lawsonia Inermis* Linn.

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Abstract: *The potency of different solvent systems on extraction of phenolic compounds from henna leaf and effect of packaging and storage conditions in retaining the quality of oven dried henna leaf powder were explored. Methanolic, ethanolic and acetone extracts of different henna leaf samples were evaluated for amount of extract obtained, total phenolic content (TPC) and lawsone content. Methanolic extract of oven dried powdered henna leaves give maximum TPC (306.35µg tannic acid equivalent/ 100g dry weight) and lawsone content (54 µg/ml) whereas fresh leaves yielded maximum amount of extract (17.28%). To study the effect of packaging on retaining the quality of henna leaf, oven dried henna leaf powder were packaged in low density polyethylene (LDPE) pouches, aluminium foil covers and paper covers and stored under ambient and low temperature storage conditions for three months. Packaging and storage of henna leaf powder in paper covers under ambient or low temperature storage conditions can retain lawsone content, 34.33µg/ml and 37.67µg/ml respectively for three months.*

Keywords: *Extraction, henna leaf, packaging, phenol content.*

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. It is a flowering plant which has been used since the Bronze Age to dye skin (including body art), hair, fingernails, leather, silk and wool. Henna is widely used in a variety of religious and social ceremonies in India. Henna leaves have bitter bad taste and used in vulnerary, diuretic, headache, hemicranias, lumbago, bronchitis, boils, ophthalmia, syphilitic, sores, amenorrhoea, scabies and spleen diseases and also favours hair growth.

The principle colouring substance of henna is a red-orange coloured molecule lawsone (2-hydroxyl-1,4-naphthaquinone), a burgundy organic compound that has an affinity for bonding with protein having molecular formula, $C_{10}H_6O_3$ and melting point of 190°C.

Lawsone is primarily concentrated in the leaves, especially in the petioles of the leaf. Whole, unbroken henna leaves will not stain the skin. Henna will not stain skin until the lawsone molecules are made available (released) from the henna leaf. Fresh henna leaves will stain the skin if they are smashed with a mildly acidic liquid. The lawsone will gradually migrate from the henna paste into the outer layer of the skin and bind to the proteins in it, creating a fast stain.

Dried henna leaves was found to be a better source of lawsone as compared to the fresh leaves [3]. Phenolic compounds, widely distributed within plants, are commonly isolated, using aqueous or organic solvents [4]. Several extraction techniques have been reported for extraction of phenolic compounds from different matrices using solvents with different polarities, such as methanol, water, ethyl acetate and petroleum ether [2].

Samarin *et al*. [7] reported that dark condition under freezing (–18°C) within 20 days is the best condition for storage of methanol extract of henna leaves and after this point there would be degradations in phenolic acids.

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So an experiment was conducted in Department of Processing Technology, College of Agriculture, Vellayani, Trivandrum with the objective of finding out a suitable solvent which could yield maximum henna leaf extract with high lawsone content and to find out the effect of different packaging materials and storage conditions on lawsone content.

MATERIALS AND METHODS

Fresh henna leaves were collected from medicinal plants garden maintained at Instructional Farm, College of Agriculture, Vellayani. The leaves are separated from the branches collected and washed thoroughly in running water, subsequently air dried, packed in polyethylene packets and stored in refrigerator (4° C) until using for experiments. Synthetic lawsone was purchased from Merck, India. All the chemicals used were of analytical grade.

The study was conducted as two parts i.e., efficiency of solvents on lawsone extraction and effect of packaging on lawsone content of henna powder.

For studying the efficiency of solvents on extraction of lawsone, fresh henna leaves (crushed), sun dried henna leaf powder and oven dried henna leaf powder [40°C for 24 hours] [1] were extracted using three different solvent systems i.e., ethanol, methanol and acetone in a soxhlet extraction apparatus. The experiment was conducted in a design CRD with nine treatment combinations (three plant material and three solvents) and three replications.

Yield of the extract in various solvent systems was recorded using a weighing balance and expressed in percentage. The TPC (Total Phenol Content) of henna leaf extracts was determined spectrophotometrically using Folin-Ciocalteu's reagent according to the method described by Lim *et al*. [6] and expressed as tannic acid equivalent per 100 gram dry weight. Quantitative estimation of lawsone content in the extracts were carried out using spectrophotometer as described by Dhiman *et al*. [3]. The henna leaf sample yielding maximum lawsone content was subjected to packaging and storage studies for a period of three months.

Henna leaf samples were packed in three different packaging systems *i.e.* LDPE (Low Density Polyethylene) covers, aluminium foil covers and paper covers. These were stored under ambient and low temperature storage (20 ± 2° C) conditions and evaluated for moisture gain and lawsone content at monthly intervals for a period of three months.

Experimental data was analysed using analysis of variance (ANOVA) and significant differences among means from triplicate analyses at p (<0.05) were determined by Duncan's Multiple range test.

RESULT AND DISCUSSION

Efficiency of Solvents on Phytochemical Extraction

Fresh henna leaves extracted using methanol gave maximum extract yield (17.28%) (Table 1). Yield obtained from sun dried or oven dried leaves when methanol or ethanol was used as solvent, found to be poor and on par. So, among the plant materials used, fresh henna leaves and among the solvents methanol is the better choice for maximum extract yield. The efficiency of methanol for yielding maximum amount of extract was proved by Hosein & Zineb [5] and Samarin *et al*. [7].

Total phenol content was found to be significantly higher for methanolic extract of oven dried henna leaf powder (306.35 µg tannic acid equivalent/ 100g dry weight) and fresh henna leaves (297.41 µg tannic acid equivalent/100g dry weight) (Table 1). Acetone extract of fresh henna leaves contains least phenol content (57.33 µg tannic acid equivalent/100g dry weight). Acetone extracts irrespective of the plant material used yields maximum total phenolic content and the same was reported by Samarin *et al*. [7].

Lawsone content was found to be significantly higher in methanolic extract of oven dried henna leaf powder (54.00 µg/ml) and lowest for ethanolic

Table 1
Efficiency of solvents on phytochemical extraction from henna leaves

Parameter	*Yield (%)*			*TPC (µg tannic acid equivalent/ 100g dry weight)*			*Lawsone content (ig /ml)*		
Samples	*Methanol*	*Ethanol*	*Acetone*	*Methanol*	*Ethanol*	*Acetone*	*Methanol*	*Ethanol*	*Acetone*
Fresh leaves	17.28[a]	13.84[b]	11.29[c]	297.41[ab]	187.97[c]	57.33[f]	37.66	15.00	21.33
Sun dried leaves	14.86[b]	6.65[e]	6.89[e]	285.94[b]	188.11[c]	94.55[e]	44.67	27.33	30.00
Oven dried leaves	12.61[b]	8.75[d]	8.55[e]	306.35[a]	148.66[d]	87.43[d]	54.00	29.67	34.33
CD (5%)	1.68	19.48	5.78						

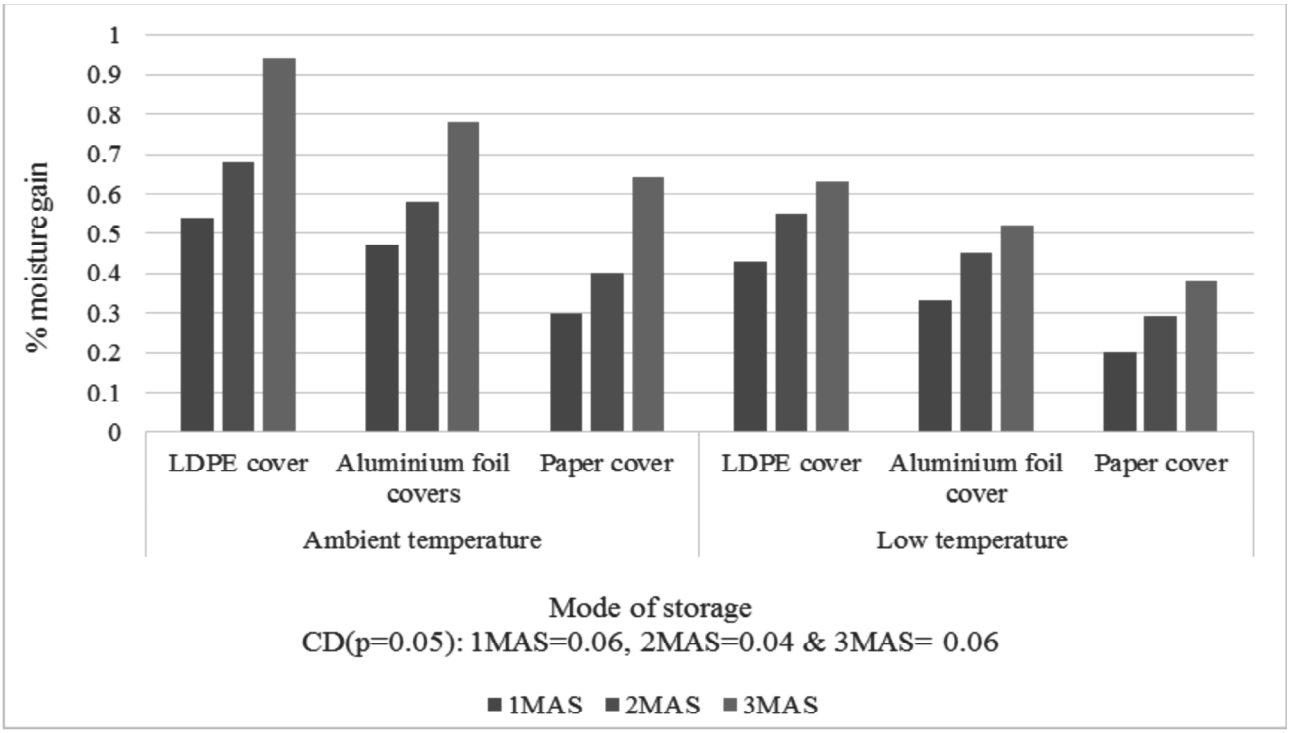

Figure 1: Moisture gained (%) by henna leaf powder during three months of storage

extract of fresh henna leaves (15.00 µg/ml) (Table 1). Oven dried henna leaf powder is a good source of lawsone than the fresh ones as reported by Dhimam *et al*. [3].

Methanol is found to be a good solvent for maximum extract yield with high total phenol and lawsone content. Oven dried powdered henna leaves yields maximum total phenols and lawsone whereas maximum amount of extract can be from fresh henna leaves.

Effect of Packaging on Quality of Henna Powder

Oven dried henna leaf powder packed in paper covers and stored under low temperature storage conditions recorded significantly lowest moisture gain (0.38%) even after three months of storage. Paper cover is identified as a good packaging material compared to LDPE covers and aluminium foil covers (Figure 1). Also, low temperature storage is ideal for henna leaf powder irrespective of the packaging material used.

Methanolic extracts were prepared from henna leaf powder packaged and stored in different storage conditions and lawsone was estimated at monthly intervals for a period of three months. At three months of storage henna leaf powder packaged in paper cover and stored under both low temperature storage (37.67µg/ml) and ambient condition (34.33µg/ml) recorded significantly higher lawsone content. Lawsone content decreased during storage period irrespective of the packaging and storage conditions and the rate of decrease was more for samples in LDPE covers stored under ambient conditions (Figure2). This may be due to the degradation of lawsone in the presence of light as reported by Samarin *et al*. [7].

CONCLUSION

Efficiency of solvents like methanol, ethanol and acetone on extracting lawsone from fresh, sun dried and oven dried henna leaves were evaluated by recording the amount of extract yielded, total phenolic content and lawsone content. Methanol was found to be good solvent for the phytochemical extraction and oven dried powdered henna leaves found to be superior in TPC (306.35µg tannic acid equivalent/100g dry weight) and lawsone content (54µg/ml). Methanolic extract of fresh henna leaves yield maximum quantity (17.28%) of extract. Oven dried henna leaf powder were packaged in LDPE covers, aluminium foil covers and paper covers and these were stored in ambient and low temperature storage conditions for evaluating the packaging and storage system suitable for retaining its quality.

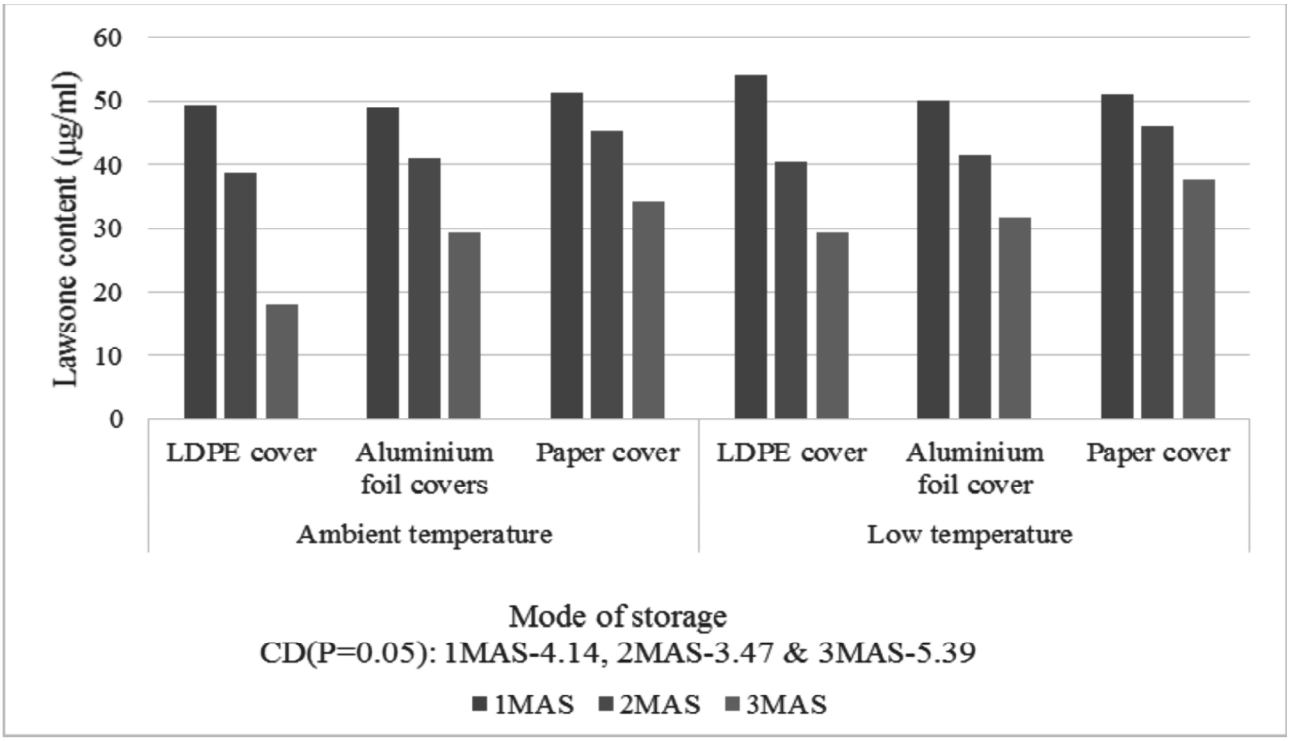

Figure 2: Lawsone content in henna leaf powder during storage

Paper was identified as a good packaging material in which oven dried henna leaf powder can be stored in ambient (lawsone content = 34.33µg/ml) as well as low temperature storage conditions (lawsone content = 37.67µg/ml) for three months without much quality loss.

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