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REGULAR ARTICLE

PHYTOCHEMICAL ANALYSIS AND INFLUENCE OF EDAPHIC FACTORS ON LAWSONE CONTENT OF *LAWSONIA INERMIS* L.

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SUMMARY

Lawsonia inermis commonly called as Henna or Mehandi is known for its cosmetic properties, and is an important source of phytochemicals of immense medicinal and pharmaceutical potential. Present study was carried out to study the influence of idaphic factors on quantitative estimation of lawsone content and morphology of leaves in Henna leaves collected from different region of Rajasthan in different season. Phytochemical analysis shows that October-November season crop leaves show good quality Henna compare to others. The Phytochemical analysis was also carried out to study the presence of different Phytochemicals in *Lawsonia inermis*. Lawsone, the active ingredient in the leaves, responsible for color production in *Lawsonia inermis* was also estimated in different plant samples.

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1. Introduction

Lawsonia inermis of family Lythracae commonly known as Henna is a Perennial shrub. It was believed to have originated in North Africa (Egypt Arid area perhaps Ethiopia) and has naturalized and cultivated in the tropics of America, Egypt, India and part of Middle East. It is also known as El-Henna, Egyptian priest, and Mignonette tree. Henna is a large shrub reaching a height of up to 6 meters. It has spreading lateral branches with opposite leaves (Simon *et al*., 1984). Leaves of henna contain Lawsone the chief active ingredient responsible for its dye properties. The dried leaves paste is used as cosmetic for decoration of hand, feet and body on different festivals and religious occasions in India, as hair dye and hair conditioner to improve their lustre (khem chand *et al*., 2003) and also extensively used as a dye in silk and wool industries. In India, Henna is mostly grown in the states of Rajasthan, Gujrat, Madhya Pradesh and Punjab. Rajasthan henna farms normally produce body art quality henna while Punjab henna is mostly used for the purpose of hair dye (Khandelwal, 2002).

In the states Gujrat, Madhya Pradesh & Punjab henna mostly used for the purpose of hair dye while Rajasthan henna farm normally produce body art quality henna. Leaves of henna contain Lawsone the chief ingredient is responsible for dye properties of henna. Normally the concentration of Lawsone founded in leaves between 0.5 to 1.5% (Simon *et al*, 1984). But physical conditions influence on the dye properties and percent of Lawsone. Henna leaves also contain mannitol, tannic acid, mucilage, gallic acid, and napthaquinone. The flower of Henna has a strong aroma with high commercial value (simon *et al*, 1984). They are intensely fragrant, and are used in perfumery. The Kannaj and Ujjain are the main manufacturing centers of perfume India. Dried leaves of henna used as a cosmetic for application on hands and feets during festivals and religious occasion in India. Henna is also used as hair conditioner to improve its lustre (khem chand *et al*, 2003).

The wide-ranging ethanomedicinal survey revealed that *L. inermis* L. is known as a magic potion in the herbal medicine with varied pharmacological activities (Upadhyay et al., 2010b). This resourceful medicinal plant is the inimitable source of various types of phytochemicals, which are responsible of the various activities of the plant. Therefore, extensive investigation is needed to exploit their therapeutic utility to combat diseases.

2. Material and Method

Collection of plant material

To study the phytochemical constituents, the collected leaves were air-dried in shade and ground into fine powder using grinder. The aqueous extract of sample prepared by soaking 100gm of dried powdered sample in 200ml of distilled water for 12h. The extract was filtered using Whatman filter paper No 42 (125mm). From different region of Rajasthan leaves are collected to studies their various parameters as given in Table 1-5. Leaves are collected from different region of Rajasthan in different growing seasons.
The samples collected are as follow: -

1. Rain-fed crop in March. – Sojat (Pali).
2. Water irrigated crop in March season -Sojat (Pali).
3. Rain-fed crop in October -Nov season-Sojat (Pali).
4. Water Irrigated crop in October-Nov. season-Sojat (Pali).
5. Normal water irrigated Jaipur region plant in March season.

Phytochemical test were carried out on the aqueous extract using standard procedures to identify the Tannin, Phobatannin, Flavanoid, steroid, Terpenoid, Cardiacglycoside (Harborne 1973; Sofowara 1993).

Methodology

Quantitative analysis of Lawsone in various Samples was done by using Colorimetric method involves the soaking of 100 mg finely leaf ground material of Henna in 10 ml of distilled water for 2hrs. Thereafter the mixture centrifuged at 5000rpm for 20minutes and the clear supernatant separate out into a test tube. The absorbance of supernatant read out at 452nm wavelength. Further a calibration curve is prepared by plotting the concentration versus absorbance at this wavelength in the concentration range 0 to 10 PPM of the pure dye. The Lawsone content in the samples was then calculated by the method of (Pratibha and Korwar, 1999).

Formula

Lawsone in sample (mg/gm) = PPM in test solution x Dilution Factor

3. Results

Quantitative analysis of lawsone was carried out from the different samples 1 to 5. Their lawsone estimation in percentage is given in tables 4, 6, 8, 10, 12 and 13. For each sample 5 replicas were taken and percent lawsone content was determined. The value for the concentration of lawsone in mg/gm was calculated on the basis of standard calibration table and curve (Table- 2, Figure 1). The winter harvest (Oct. - Nov. leaves, sample 4 and 5) had more lawsone contents (Table- 9 to 12) compared to spring season harvest (sample 1 to 3) (Table- 3 to 8).

The rain-fed crop showed more percent lawsone contents in both the seasons spring and winter (sample 1and 4) (2.49% and 2.99% respectively) (Table- 4 and 6) over irrigated field samples (sample 2 and 5) (2.39% and 2.586% respectively) (Table- 6 and 12). The lawsone content was highest in rain-fed crop samples of winter season i.e. 29.9 mg/gm (Table- 10). Among the irrigated crop of spring season from Sojat and Jaipur, it was observed that Sojat henna crop had more lawsone contents (23.9%) (Table- 6) over Jaipur (19.9 mg/gm) (Table- 8). It is clear from the results that Sojat henna crop has more lawsone contents in their leaves compare to Jaipur henna leaves.

The comparative study of Lawsone contents in Sojat henna crops in spring and winter, between rain-fed and irrigated conditions; it is clear that the rain-fed condition induce more of lawsone contents then the irrigated crop (Table- 4, 6, 8, 10, 12 and 13). The minimum lawsone contents

from the Sojat crop was 23.9 mg/gm in irrigated crop of spring. However the overall the percentage of lawsone ranged between 2.39 to 2.99%. Therefore, to have maximum lawsone contents in henna crop it should be under stress condition and not flood irrigated.

Table-1: Qualitative analysis of phytochemical constituents of henna leave

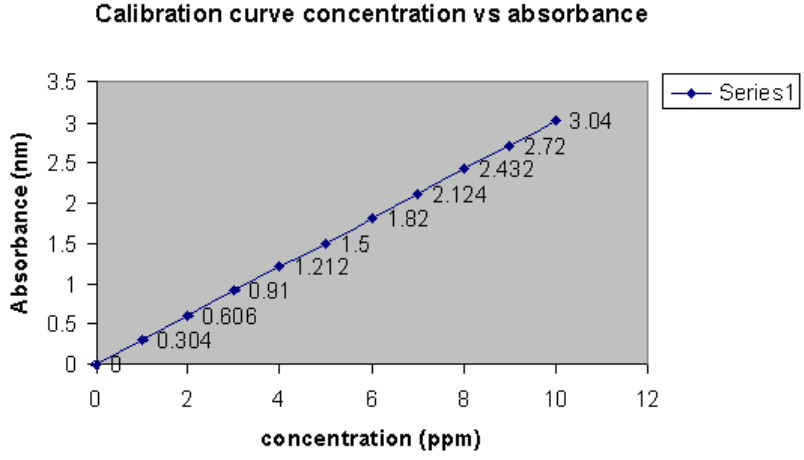
Tannin	Phobatannin	Gallic Acid	Saponins	Naptha-quinone	Flavanoid	Steroids	Terpenoid	Cardio-glycosides
++	+	++	–	+++	+	+	++	+

Quantitative estimation of Lawsone

Table-2: Standard data table of Calibration curve

S.No.	Concentration (PPM)	Wavelength (nm)	Absorbance
1	1	452	0.304
2	2	452	0.606
3	3	452	0.910
4	4	452	1.202.
5	5	452	1.500
6	6	452	1.820
7	7	452	2.124
8	8	452	2.432
9	9	452	2.720
10	10	452	3.040

Fig. 1: calibration curve of absorbance and concentration in ppm



Morphological studies of leaves

Table-3. Rainfed crop in March season –Sojat (Pali). (Fig. 2)

S.no.	Leaves	Size (Length + width) in cm
1	Apical Leaves	1.5+0.7
2	Middle Leaves	2.0+1.0
3	Mature Leaves	3.4+1.4
4	No. of Leaves / Branch	55-60
5	Attachment of Leaves	2 Leaves/Internode
6	Color of Leaves	Dark Green
7	Branch Length	70-75 cm

Table-4. Lawsone estimation from Rain-fed crop of March season leaves of Sojat (sample 1)

Sample	Replica	Absorbance (nm)	PPM Value	Concentration (mg/gm)	Lawsone Percentage	Average Lawsone (%)
Rain fed crop leaf samples	1	0.765	2.53	25.3	2.53	
	2	0.740	2.45	24.5	2.54	
	3	0.755	2.50	25.0	2.50	2.49±0.034
	4	0.770	2.54	25.4	2.54	
	5	0.745	2.46	24.6	2.46	

Table-5. Water irrigated crop in March season –Sojat (Pali). (Fig. 3)

S. no.	Leaves	Size (Length + width) in cm
1	Apical Leaves	1.5+0.8
2	Middle Leaves	2.5+1.4
3	Mature Leaves	5.4+2.4
4	No. of Leaves / Branch	50-60
5	Attachment of Leaves	2 Leaves/Internode
6	Color of Leaves	Dark Green
7	Branch Length	60-70 cm

Table-6. Lawsone estimation from water irrigated crop of March season leaves (sample-2) of Sojat

Sample	Replica	Absorbance (nm)	PPM Value	Concentration (mg/gm)	Lawsone Percentage	Average Lawsone (%)
water irrigated crop leaf sample	1	0.724	2.39	23.9	2.39	
	2	0.732	2.42	24.2	2.42	
	3	0.718	2.37	23.7	2.37	2.39±0.02
	4	0.720	2.38	23.8	2.38	
	5	0.728	2.41	24.1	2.41	

Table-7. Normal water irrigated Jaipur region plant in March season. (Fig. 4)

S.no.	Leaves	Size (Length + width) in cm
1	Apical Leaves	2.2+1.1
2	Middle Leaves	4.0+1.8
3	Mature Leaves	6.0+2.4
4	No. of Leaves / Branch	32-36
5	Attachment of Leaves	2 Leaves/Internode
6	Color of Leaves	Dark Green
7	Branch Length	55-60

Table-8. Lawsone estimation from water irrigated crop of March season leaves (sample-3) of Jaipur

Sample	Replica	Absorbance (nm)	PPM Value	Concentration (mg/gm)	Lawsone Percentage	Average Lawsone (%)
water irrigated crop leaves samples	1	0.608	2.01	20.1	2.01	1.99±0.02
	2	0.598	1.98	19.8	1.98	
	3	0.597	1.97	19.7	1.97	
	4	0.612	2.02	20.2	2.02	
	5	0.605	2.00	20.0	2.00	

Table-9. Rain fed crop in October –November season –Sojat (Pali). (Fig. 5)

S.no.	Leaves	Size (Length + width) in cm
1	Apical Leaves	2.7+1.4
2	Middle Leaves	3.7+1.5
3	Mature Leaves	5.6+1.9
4	No. of Leaves / Branch	40-50
5	Attachment of Leaves	2-3 Leaves/Internode
6	Color of Leaves	Light yellow-Green
7	Branch Length	55-65

Table-10. Lawsone estimation from Rain fed crop of October- November season leaves (sample-4) of Sojat

Sample	Replica	Absorbance (nm)	PPM Value	Concentration (mg/gm)	Lawsone Percentage	Average Lawsone (%)
Rain fed crop leaf samples	1	0.908	3.00	30.0	3.00	2.99±0.02
	2	0.912	3.01	30.1	3.01	
	3	0.898	2.97	29.7	2.97	
	4	0.914	3.02	30.2	3.02	
	5	0.898	2.97	29.7	2.97	

Table-11. Water Irrigated crop in October-November season –Sojat (Pali). (Fig. 6)

S.no.	Leaves	Size (Length + width) in cm
1	Apical Leaves	3.0+1.6
2	Middle Leaves	4.0+1.8
3	Mature Leaves	5.6+2.5
4	No. of Leaves / Branch	24-30
5	Attachment of Leaves	2 Leaves/Internode
6	Color of Leaves	Dark Green
7	Branch Length	75-80

Table-12. Lawsone estimation from water irrigated crop of October-November season leaves (smple-5) of Sojat

Sample	Replica	Absorbance (nm)	PPM Value	Concentration (mg/gm)	Lawsone Percentage	Average Lawsone (%)
water irrigated crop leaf samples	1	0.779	2.57	25.7	2.57	
	2	0.770	2.54	25.4	2.54	
	3	0.802	2.65	26.5	2.65	2.58±0.04
	4	0.789	2.61	26.1	2.61	
	5	0.775	2.56	25.6	2.56	

Table-13. Concentration and Percentage of Lawsone in different harvesting seasons plant leaves

Sample No.	Samples	Average Lawsone Contents (mg/gm)	Percent Lawsone (%)
1	Rainfed- Spring -Sojat	24.90	2.49±0.03
2	Irrigated-Spring-Sojat	23.90	2.39±0.02
3	Irrigated-Spring-Jaipur	19.90	1.99±0.02
4	Rainfed-winter-Sojat	29.90	2.99±0.02
5	Irrigated-winter-Sojat	25.86	2.58±0.04

Morphology of Leaves of different locations

Fig. 2

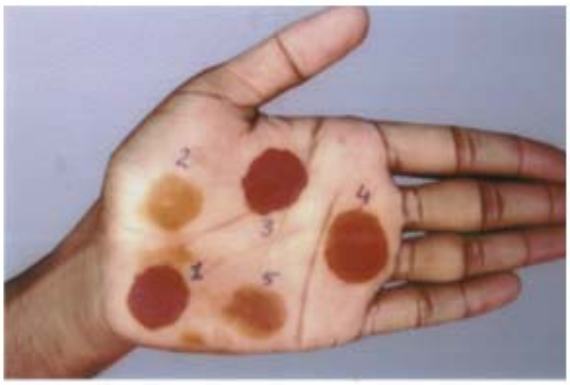
Fig. 3

Fig. 4

Fig. 5

Fig. 6

Figure-7. Showing dye spot of different region leaves



4. Discussion

The wide-ranging ethanomedicinal survey revealed that *L. inermis* L. is known as a magic potion in the herbal medicine with varied pharmacological activities. This resourceful medicinal plant is the inimitable source of various types of phytochemicals, which are responsible of the various activities of the plant. The Phytochemical study of Lawsonia *inermis* L. showed that the leaves of henna were rich in Tannin, Flavanoid, Terpenoid, Steroid, Cardiac glycoside, 2, Hydroxy 1-4, Napthaquinone. They were known to show herbal medicinal and herbal cosmetics properties.

Our results revealed that color, number and leaf size showed morphological variation in the Lawsonia inermis populations; these are strongly influenced by environmental factors. morphological variation is apparently the result of an adaptive response to the environment; for example, variation in growth traits and phonological traits is associated with a latitudinal and altitudinal range or by contrasting climatic conditions. The observed trend of morphological variation made mention of adaptation to the contrasting micro-edaphic conditions prevailing for these groups and this was supported by the significant correlation with soil physicochemical characteristics. The greater discrimination power of adaptation micro edaphic conditions compared to the geographical regions of origin of accession in this study clearly indicated the greater importance of environmental factors (soil texture, soil chemical characteristics, and annual rainfall) than geographical location, in discriminating populations. Human actions and natural selection factors, by affecting morphological traits related to adaptation of a population, could allow interference with adaptation due to genetic distances from quantitative traits.

The quantitative estimation of different region plant leaves shows the variation in the Active Ingredient (Lawsone). The March season leaves contain less Lawsone contents compare to October –November season plant leaves. The qualitative analysis of each sample was also done by applying their paste on hands. The result shows that the October-November season leaves paste taken good color on hands then other season crop leaves. The result concluded that the lawsone alone is not responsible for dyeing properties

of Henna but presence of other compounds along with its add its dye quality for e.g. Tannin, Mucilage, Gallic acid, etc. Stress conditions may increase the dye properties of Henna.

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