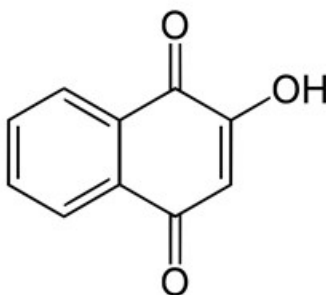
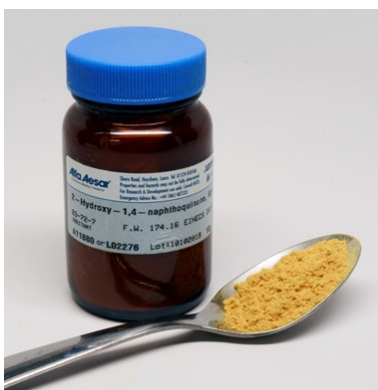


Chapter 4: Science and Microscopy

Part 2: The Phytochemistry of Henna, *Lawsonia inermis* L. [Lythraceae]

Lawsonone: 2-hydroxy-1,4- naphthaquinone and its precursors

Some henna hair dye companies have claimed that all of the various colors of their henna hair dye come from henna and henna only; that red henna was from leaves, black henna was from henna roots, and brown henna was from henna bark. This was not, and cannot have been true.¹ Lawsonone is the only dye molecule produced from henna in a significant amount and it is only produced from the leaves. There is no other dye in any other part of the henna plant that will dye hair, and henna stains keratin only in the range of copper to dark auburn. Though some exporters claim to be breeding blue, purple, pink, and other colors of henna, there is no evidence of such thus far.



Left: Lawsonone: 2-hydroxy-1,4- naphthaquinone as a 98% pure powdered chemical.²

Middle: Lawsonone molecule

Right: henna leaves which contain the *precursors* to lawsonone, 2-hydroxy-1,4- naphthaquinone exist in henna leaves as hennosides, releasing about 1% lawsonone.

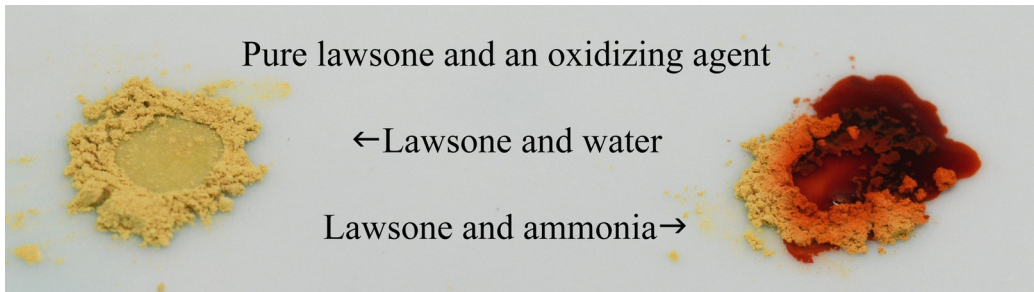
In its pure state, lawsonone is an orange molecule, with the specifications:³ 2-Hydroxy-1,4-naphthoquinone, 98+%. MDL: MFCD00001678. EINECS: 201-496-3. Formula: C₁₀H₆O₃. Formula Weight: 174.16 Melting point: ca 193° dec. Storage & Sensitivity: ambient temperatures. Solubility: Soluble in water: 2 g/L at 20°C. Lawsonone is produced when released from the hennocide precursors in the henna leaf through testing and application.⁴

¹ This may have been a convenient lie to circumvent the FDA regulation that henna is the only plant unconditionally permitted to dye hair, or it may have been to keep competitors and consumers ignorant of what was actually in the box.

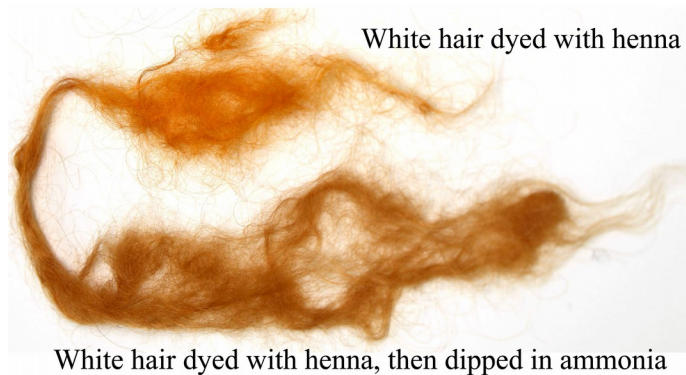
² Alfa Aesar: Applications for 2-Hydroxy-1,4-naphthoquinone is used for preparing decorative hair and skin dyes. 2-Hydroxy-1,4-naphthoquinone also demonstrates antimicrobial and antioxidant effects. It also suppresses the formation of hydrogen peroxide and superoxide radical anion by aldehyde oxidase-catalyzed reactions.

³ Alfa Aesar, Thermo Fisher Scientific

⁴ Gallo, F., Multari, G., Palazzino, G., Pagliuca, G., Zadeh, S. M. M., Biapa, P. C. N., Nicoletti, M.; 2014. "Henna through the Centuries: A quick HPTLC analysis proposal to check identity." *Revista Brasileira de Farma Cognisia*. 24, 2



Lawsone is vulnerable to oxidation. Oxidation can come from exposure to air over time, or to strong oxidizing agents in a matter of minutes. People who use henna to dye their hair or to ornament their skin are familiar with the effect of an alkaline on henna stain. People who perspire profusely often find that their henna stain shifts from red-orange to brown or even black. People whose tap or well water is alkaline from mineral content find the bright henna tones in their hair darken over time. This can be mitigated with antioxidants and may be reversed with Ancient Sunrise® Rainwash.⁶



An oxidant will push henna stain from reddish orange to a brown color.

The formation of lawsone from precursors in the henna leaf by acidic hydrolysis

Lawsone is produced from hennocide precursors in the henna leaf. “Conversion of the three isomerglucosides (hennosides) into the unique aglycone by hydrolysis in mildly acid conditions. Further transformation of the aglycone leads to the more stable lawsone by easy oxidation.”⁷ As described by Gallo, Multari, et al, the sequence of henna dye release and binding is as follows:

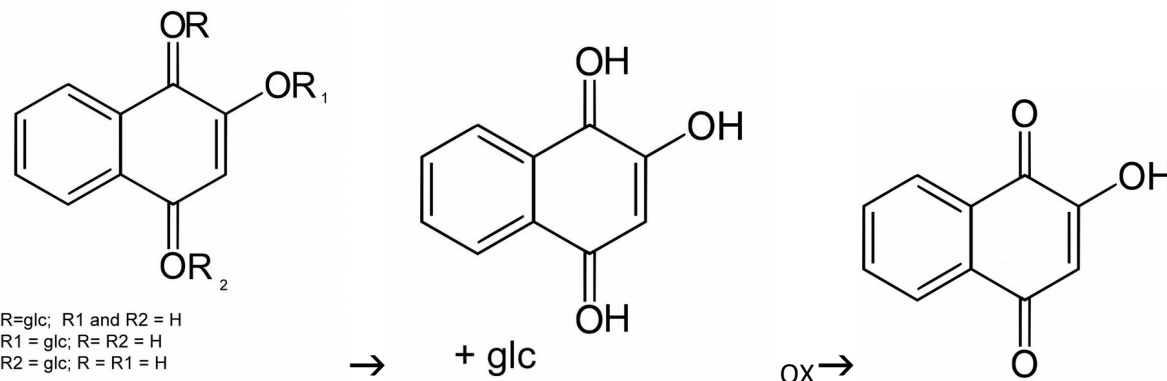
“Lawsone is not present as a free molecule in the leaves, but it is derived from its precursors, the hennosides, during henna preparation. Hennosides are three isomers

⁵ 98% pure lawsone was measured in 2 samples. 4 drops of dilute lemon juice were applied to the sample on the left, and 4 drops of ammonia were applied to the sample on the right.

⁶ Ancient Sunrise® Rainwash: http://www.mehandi.com/Ancient-Sunrise-Rainwash-Mineral-Treatment-p/rain_01.htm Citric Acid, Ascorbic Acid, Xanthan Gum

⁷ Gallo, F., Multari, G., Palazzino, G., Pagliuca, G., Zadeh, S. M. M., Biapa, P. C. N., Nicoletti, M.; 2014. “Henna through the Centuries: A quick HPTLC analysis proposal to check identity.” *Revista Brasileira de Farma Cognisia*. 24, 2

derived from the tautomeric forms of the keto-enol interconversion of the naphthoquinone structure. In this case the second ring is thrice oxygenated, that can give rise to three possible hydroxyl groups and consequently change to the diketonic form. Each of the hydroxyls can be glucosidated, giving rise to the three isomers. The aglycone, derived from their hydrolysis, is further converted by oxidation into lawsone that is the dyeing active compound.”⁸



Lawsone is produced from hennocide precursors in the henna leaf.

In less technical terms, lawsone’s dye release from henna is facilitated when the powdered henna leaf material is mixed with a mildly acidic medium; a pH 5.5 paste mix is ideal. In this mildly acidic pH paste, the lawsone molecule can be released and migrate from henna paste to stain keratin.⁹ A Michael Addition facilitates a non-fading stable bond of the lawsone molecule with keratin. This red-orange stain can gradually oxidize to a brownish color when bound with keratin. In alkaline conditions, the stain can oxidize to black or greenish black.

The precursor is converted into the intermediate aglycone by hydrolysis in mildly acidic conditions. The aglycone intermediates will bind to keratin. Neither the precursor nor the final lawsone will bind as effectively to keratin as the aglycone intermediate.¹⁰ In the mildly acidic henna paste at room temperature, the aglycone will become available after about an 8 hour soak, and remain at maximum in the paste for 12 – 24 hour hours, after which the percentage of the bindable aglycone form of the lawsone molecule will gradually diminish. This is termed ‘demise’ of the henna paste; at this point the henna paste produces diminishing stains. This transformation is gradual at room temperature. This sequence proceeds more quickly in warm conditions and slows under cold conditions.

The acidic paste maintains the hydrogen atoms on the corners of the aglycone, the intermediate form of the lawsone molecule. In acidic mixes of henna, the intermediate form of lawsone will migrate into the keratin in hair or skin and darken as it binds permanently with the keratin by a

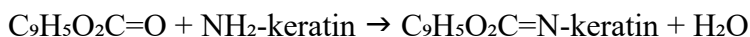
⁸ Gallo, F., Multari, G., Palazzino, G., Pagliuca, G., Zadeh, S. M. M., Biapa, P. C. N., Nicoletti, M.; 2014. “Henna through the Centuries: A quick HPTLC analysis proposal to check identity.” *Revista Brasileira de Farma Cognisia*. 24, 2

⁹ Amro, I. H., James, K. C, and Turner, T. D.; 1993. “A Quantitative Study of Dyeing with Lawsone,” *Journal of the Society of Cosmetic Chemistry*, 45, 159 - 165

¹⁰ Attempting to improve henna powder by adding lawsone powder will not improve the henna stain. Only the intermediate aglycone can effectively stain the hair.

Michael Addition.¹¹ If the henna powder is mixed only with water, the hydrogen atoms are not as well conserved. Henna mixed with water is more likely to fade from air because unbound lawsone will gradually wash out of hair. Henna mixed with a mildly acidic mix will leave a stain in hair that is not only permanent, but will gradually darken, and continue to darken for years.

The sequence of lawsone migrating from henna to bind with protein in the outer layer of skin or hair is as follows according to Dalgies, 1949.¹²



All of the unstable aglycones in henna paste usually transform to the stable state of lawsone in about one week at room temperature in the presence of oxygen. In henna work, this is referred to as demise. This demised henna paste stains keratin a weak orange color which will not darken because it can no longer bind through Michael Addition.

A comparison test showing importance of the precursors to the stain

One might wonder why pure lawsone is not a popular hair dye product, given people's reluctance to spend the time and mess required to dye hair with henna. One might wonder why people do not simply paint their skin with lawsone to adorn their skin. The reason would be that pure lawsone does not contain the precursors, and without them, the binding to keratin is greatly decreased and stain results diminished.



This photograph compares naturally white hair dyed for 12 hours with 98% pure lawsone, and henna with 1.5% lawsone, both mixed in a mildly acidic medium, photograph taken three days after application and rinsing.

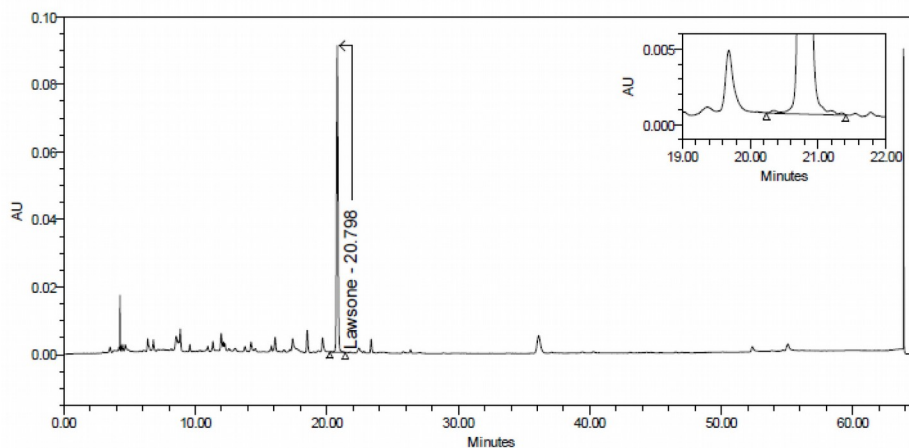
¹¹ Semwala, R. B., Semwala, D. K., Combrinck, S., Cartwright-Jones, C., Viljoen, A. (2014) "Lawsonia inermis L. (henna): Ethnobotanical, phytochemical and pharmacological aspects." *Journal of Ethnopharmacology*, June 2014

¹² Dalgies, C.E., (1949) "Naphthoquinone antimalarials. Mannich bases derived from Lawsone." *The Journal of the American Chemical Society*. 71, 1697-170

¹³ Pure lawsone was mixed with lemon juice and water, the henna was mixed with lemon juice. The hair is natural white mohair, unbleached and undyed. The hair was left to soak in the lawsone and the henna mix for 12 hours, then rinsed.

In the preceding image, the dye results of 98% pure lawsone is compared to the result of 1.5% lawsone henna paste on hair;¹⁴ the difference is dramatic. The 1.5% lawsone from henna leaf precursors creates a vivid copper-red stain on white wool fiber whereas purified 98% lawsone which does not contain the precursors creates a weak apricot-orange stain. In addition, over time, the pure lawsone fades, and the henna stain darkens. This shows that presence of the lawsone precursor in henna is crucial for the dye uptake. A hair dye or skin dye made simply of pure lawsone would be ineffective and expensive,¹⁵ as well as potentially hazardous.¹⁶

Measuring lawsone released from henna



HPLC test results of henna, 1.3 % lawsone¹⁷

HPLC (High Performance Liquid Chromatography) tests of powdered henna can be used to precisely calculate the amount of lawsone released from henna leaves. In HPLC tests done by Alkemist Laboratories, warm distilled water sonification¹⁸ acts as a dye release so the released lawsone is calculated. The HPLC tests done on henna for Ancient Sunrise®, mehandi.com typically show 1.3% to 2.7% lawsone.¹⁹ Poor quality henna may have 0.3% lawsone. A greater amount of lawsone measured in an HPLC test correlates positively to the saturation of color in hair: a higher lawsone content henna will give more vivid color on graying hair, and a lower lawsone content will deposit less color to hair.

¹⁴ Pure natural washed white mohair is used in this case

¹⁵ A henna exporter offered to add pure lawsone powder to henna when I showed him the test results that his henna was only 0.07% lawsone. Adding pure lawsone to henna powder would make little, if any difference in the outcome of the color.

¹⁶ Santa Cruz Biotechnology, Inc. Material Safety Data Sheet 2-Hydroxy-1,4-naphthoquinone sc-254254. <http://datasheets.scbt.com/sc-254254.pdf>

¹⁷ HPLC laboratory results, Alkemist Laboratories for TapDancing Lizard LLC 2013

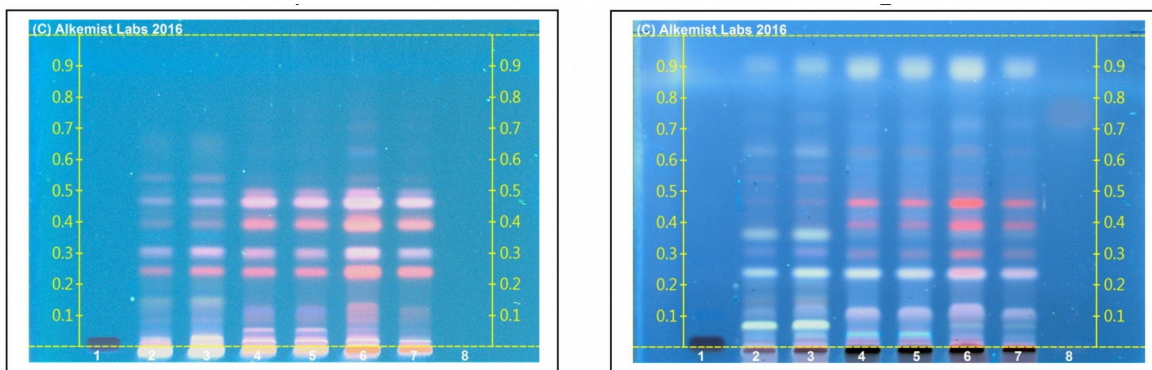
¹⁸ Sample preparation: 450 mg henna powder into a 25 mL volumetric flask; added 20 mL water and sonicated for 30 minutes at 45 °C. Allowed to cool to room temperature and villed to volume with water and made a 5:50 dilution in water. Centrifuged for 10 minutes. Transferred to HPLC vial for analysis.

¹⁹ HPLC laboratory results, Alkemist Laboratories for TapDancing Lizard LLC, 2008 - 2016

The fineness of sift does not seem to affect lawsone content.²⁰ There can be a negative correlation between the fineness of sift and lawsone content in the cases where the speed of the milling heats the henna; milling machinery that is slower, or which is artificially cooled appears to preserve lawsone content.

Lawsone content can also be extracted and measured from henna leaves with hexane, dichloromethane, and 95% ethanol, respectively, until exhaustion using soxhlet apparatus.²¹ This test does not necessarily yield identical results to the water sonification test. The same testing procedure should be used on every batch of henna to ensure an accurate comparison between batches.

An HTPLC (High-performance thin-layer chromatography) test can be used to evaluate samples of alleged henna hair dye powders of unknown content, which may or may not contain henna and other adulterants. In an examination of eight commercial henna powders and two collected henna leaves, samples showed considerable variation in lawsone concentration ranging from 0.004 up to 0.608 wt%, indicating that some “henna hair dye” samples were almost devoid of lawsone,²² and at best, these products meant as hair dye had very low lawsone content.



HTPLC test for lawsone²³

An HTPLC test for lawsone does not measure the amount of lawsone as precisely as does an HPLC test but it does measure the presence or absence of lawsone. In the above HPTLC photo documentation,²⁴ Lanes 2 and 3, 6 and 7²⁵ are reference samples; lanes 4 and 5 are a sample of pure, unadulterated henna powder.

²⁰ One henna exporter claimed that their henna is nearly 100% lawsone because it was triple sifted, claiming that because each time the henna was sifted, the lawsone content would be higher. This is not the case. The allegedly “near 100% lawsone henna was 1% lawsone.

²¹ Charoensup, R., Duangyod, T., Palanuvej, T., and Ruangrungs, N. (2017) “Pharmacognostic Specifications and Lawsone Content of Lawsonia inermis Leaves” *Pharmacognosy Research* Jan-Mar; 9(1): 60–64.

²² El-Shaer, N.S., Badr, J. M., Aboul-Ela, M. A., Gohar, Y. M. (2007) “Determination of lawsone in henna powders by high performance thin layer chromatography.” *Journal of Separation Science* 2007 Dec 30 (18): 3311-5.

²³ HPLC laboratory results, Alkemist Laboratories for TapDancing Lizard LLC 2013

²⁴ HPLC laboratory results, Alkemist Laboratories for TapDancing Lizard LLC

²⁵ Reference lanes for henna from The *Ayurvedic Pharmacopeia of India* Part I Volume II

Other commonly occurring compounds, contaminants, additives and adulterants in henna

Henna leaves contain chlorophyll, fiber, tannins, flavonoids, and the other phytochemicals that one might normally expect in the leaves of a small deciduous tree. Though there is no substantial amount of any dye produced by henna other than lawsone, there are other variable characteristics in henna that a person dyeing their hair or ornamenting their skin will be able to observe without a microscope or lab test.

The naphthoquinones in henna have received the greatest research attention, as the dye properties, antimicrobial, and strong antioxidant effects proceed from these. Henna contains a number of naphthoquinones including 2-methoxy-3-methyl- 1,4-naphthoquinone and lawsone (2-hydroxy-1,4-naphthoquinone). These henna naphthoquinones are derived from naphthalenes. A small number of naphthalenes, lawsoniaside (1,2,4-trihydroxynaphthalene-1,4-di- β -D-glucopyranoside) 1,2,4-trihydroxynaphthalene-1-O- β -D-glucopyranoside three methyl naphthalene carboxylates, lawsonaphthoate A-C, and 1,2-dihydroxy-4-Oglucosyloxynaphthalene have been isolated from henna stems and leaves.²⁶

In addition to quinones and naphthalenes, henna leaves contain flavonoids. These flavonoids present in henna leaves are acacetin, acacetin-7-O-glucoside, luteolin, luteolin-7-O-glucoside, apigenin-7-O- β -D-glucopyranoside, apigenin-40-O- β -D-glucopyranoside, luteolin-30-O- β -Dglucopyranoside, apiin, cosmosiin, isoscutellarin, lawsochrysin, lawsochrysinin, lawsonaringenin, 30,40-dimethoxyflavone, 7-hydroxyflavone, 3,30,40,7-tetrahydroxyflavanone and rhoifolin.²⁷ These flavonoids may be responsible for the slight variation in henna stain results among cultivars.



Mucilaginous “stringy” henna from Yemen

Henna leaves contain varying amounts of mucilage, a polar glycoprotein and an exopolysaccharide. Mucilage is produced to one extent or another by nearly all plants, and is abundant in okra, aloe vera, and flax seeds. For people who use henna, mucilage is what gives

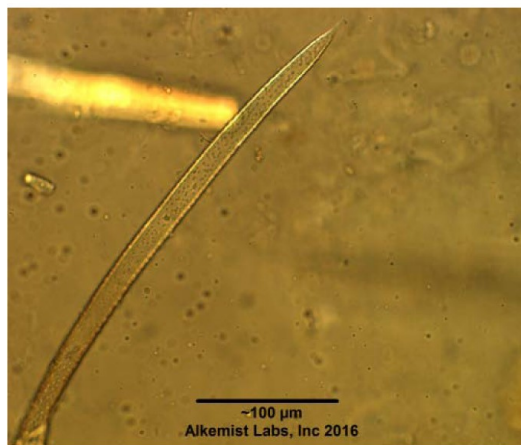
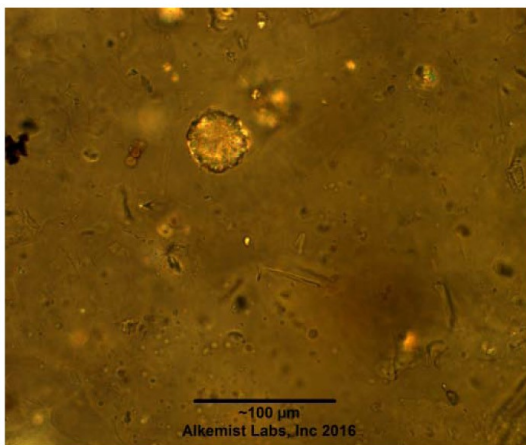
²⁶ Semwala, R. B., Semwala, D. K., Combrinck, S., Cartwright-Jones, C., Viljoen, A. (2014) "Lawsonia inermis L. (henna): Ethnobotanical, phytochemical and pharmacological aspects." *Journal of Ethnopharmacology*, June 2014 p 7.

²⁷ Ibid

²⁸ Photograph of “stringy” henna paste by Catherine Cartwright-Jones PhD

some henna the characteristic of gooiness which makes the paste as stringy as hot mozzarella cheese. In my experience, the cultivars of henna from Yemen have higher mucilage content than henna cultivars from Rajasthan, and henna cultivars from Pakistan have the lowest mucilage content. Mucilage has no effect on the dyeing action of henna in hair, but some people who use henna for body art find stringy, mucilaginous paste more difficult to manipulate. Henna paste with little mucilage is more prone to cracking and falling off the skin, so those who use henna for body art often add some form of sugar to the paste, monosaccharides being more conducive to smooth paste than polysaccharides.

Calcium oxalate is present in over a thousand²⁹ plants,³⁰ and is present at varying levels in henna.³¹ In samples I have tested, higher levels of calcium oxalate occur in henna from Yemen and lower levels in henna from Rajasthan and Pakistan. Calcium oxalate does not affect henna as a hair dye. In body art, an acidic mix of henna with high levels of calcium oxalate becomes grainy 12 – 18 hours of initial mixing, and makes henna paste difficult to manipulate after that: the crystals dissolve in the acid and change the texture of the paste. Calcium oxalate does not penetrate the skin through scalp or body art, so external applications of henna with higher levels of the crystals will not increase the probability of or accumulation of kidney stones. Henna should not be ingested or used internally; needle-shaped calcium oxalate crystals can be very irritating to mucous membranes, causing numbness and burning sensation of the tongue, mouth, and lips and swelling of tongue or lips.



32

Microscopy of henna mesophyll showing fragments of calcium oxalate crystals, including a needle-like calcium oxalate crystal.

There is inevitably some amount of sand found in henna powder, though certainly not actually produced by the henna plant. Henna grows in semi-arid zones, and removing all of the sand can be nearly impossible, though green-dyed sand is an adulterant. This is known as "polishing in the Indian henna industry. The presence of green dye is irrelevant to the stain. Henna with this

²⁹ Francesci, V.R.; Nakata (2005). "Calcium oxalate in plants: formation and function". *Annual Review of Plant Biology* (56): 41–71.

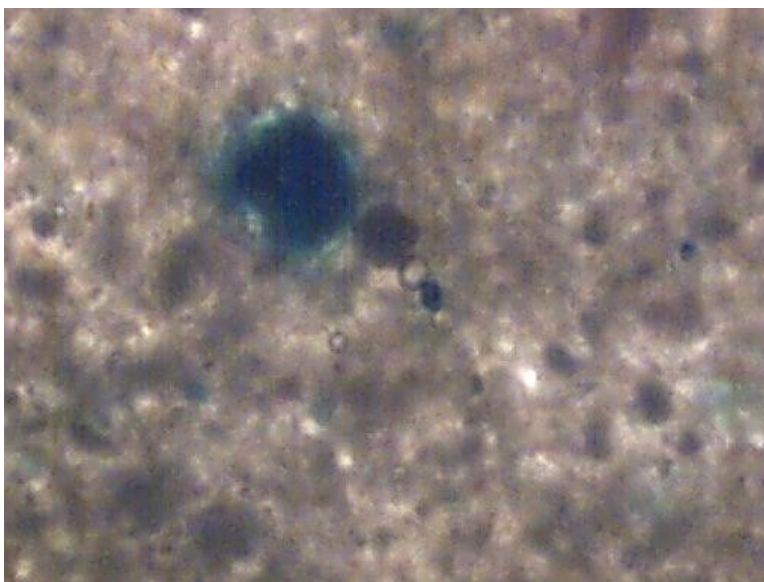
³⁰ Calcium oxalate exists in starfruit, rhubarb, beetroot, spinach and amaranth

³¹ Microscopy: Alkemist Laboratories for TapDancing Lizard LLC 2012 - 18

³² Microscopy: Alkemist Laboratories for TapDancing Lizard LLC 2016

green dye is often sold as being "fresher" and "higher quality", though the presence of green dye in henna indicates neither freshness nor quality.

The addition of green dyed sand to henna was detailed in a paper published in India challenging the marketing claim that "greener henna is better henna." The following microscopy is of henna powder with bottled lemon juice added, stirred, placed on a microscope slide, allowed 5 minutes to set, then photographed at 60 x through the microscope. The vivid green chunk shown in the center of the slide on this page illustrates the presence of an artificial green dye, a coal tar-derived dye, added to make the product more "eye-appealing." The Essential Oil Association of India investigated the green dye and published the statement,



Henna "polished" with green-dyed sand

"Major contaminants /adulterants in henna leaves are stems, dirt, plant waste and other leaves. However in case of henna powder admixture of dyed sand is observed. It has been reported that for adulteration, finely ground local sand is used. It is first dyed with auramine yellow (C.I. No. 41000) and then green with diamond green (C.I. No. 20440). This is then mixed with pure henna powder. The extent of adulteration is variable in accordance with the price of the powder reflected therein.

"Added azo dyes were not found in henna leaf samples, but yellow and green coal tar dyes were observed in powdered samples. As mentioned earlier, this may be due to the presence of (the afore mentioned dyed sand). Unlike lawsone, the natural color of henna, these added synthetic azo-dyes used for dyeing the sand or for polishing the leaves may have an adverse effect on the skin. It is, therefore, necessary to ensure that these artificial dyes are not there in the product marketed."³³

³³ Chourasia, Sardar, Patil, Mathew, and Kanpur, (1989) "Study of Quality Characteristics of Henna", *Essential Oil Association of India*

Some minerals and metals naturally occur in henna, as are in every plant and animal on earth. Ancient Sunrise® henna contains only what occurs naturally in the plant leaves because it is plant leaves and nothing else. People who are concerned about “heavy metals” being in henna have likely heard rumors that began when compound hennas with added metals were misrepresented as unadulterated henna. The various additives and adulterants used by companies that sold ‘henna hair dye’ are covered in “Ancient Sunrise Henna for Hair Chapter 3: Compound Henna.”³⁴

The minerals and metals naturally occurring in henna are generally consistent with the soil and water they grow in and other trees and bushes: nitrogen, phosphorous, magnesium and potassium. Ancient Sunrise® is tested with a heavy metals panel to assure people of their safety.

Lead acetate progressive dyes are often confused with, and marketed as henna. This misinformation may be the reason some people believe henna contains lead. No pure henna contains lead and Ancient Sunrise® henna certainly does not. Varying amounts of lead occur in soil, and therefore also in plants which grow in soil.³⁵

Ancient Sunrise® henna shipments have about level of lead as vegetables considered safe to eat, so the henna is harmless for applying to skin. The following are an example of a lead test performed by Silliker³⁶ on a batch of Ancient Sunrise® henna:

ICP-MS Sample Prep Acid Digest - EPA 3050b 6/29/15 CHG
Lead 0.29 ppm (w/w) EPA 3050/6020 USP730³⁷

People are also concerned about rumors of other heavy metals in henna, probably from the same confusion with compound henna and progressive dyes. Ancient Sunrise® henna has full heavy metal testing panels by an independent third party certified laboratory. These tests show that the levels of heavy metals found are in the same range as with a healthy plant growing on an organic farm with no additional irrigation. Ancient Sunrise® henna has the same low, safe, but measurable levels of heavy metals that exist in water, fruit, and vegetables deemed safe for consumption.

³⁴ Catherine Cartwright-Jones PhD (2018) Ancient Sunrise® Chapter 3 “Compound Henna”
http://www.tapdancinglizard.com/AS_henna_for_hair/3_compound_henna.pdf

³⁵ Thomas , B., Roughan, J. A., Watters, E. D. (1972) “Lead and cadmium content of some vegetable foodstuffs”
Journal of the Science of Food and Agriculture, Volume 23, Issue12, December 1972, Pages 1493-1498

An analytical method and results are given for the determination of lead and cadmium in Brussels sprouts, apples, pears, cabbages, potatoes, onions, leeks, carrots, swedes, watercress, frozen vegetables, cucumber, celery, tomatoes, mushrooms and dried herbs. The lead content of the 231 samples was in the range 0.01 to 3.85 parts/million the mean being 0.05 parts/million; the range and mean of the cadmium content were 0.01 to 0.22 and 0.04 parts/million, respectively.

³⁶ SILLIKER, Inc. Southern California Laboratory Merieux NutriSciences (2015) report on JAR16615TD1_3:051915HRUT Client: TapDancing Lizard, LLC

³⁷ Thomas , B., Roughan, J. A., Watters, E. D. (1972) “Lead and cadmium content of some vegetable foodstuffs”
Journal of the Science of Food and Agriculture, Volume 23, Issue12, December 1972, Pages 1493-1498

The following are an example of the results of a heavy metal panel performed by Silliker³⁸ on a batch of Ancient Sunrise® henna:

ICP MS Heavy Metals (4 analytes) EPA 3050/6020 USP730 8/3/17 CHG
ICPMS Prep Acid Digest -
Arsenic 0.45 ppm (w/w)
Cadmium 0.058 ppm (w/w)
Lead 1.48 ppm (w/w)
Mercury 0.029 ppm (w/w)

An arsenic level of 1 ppm is considered safe in drinking water by the EPA; Ancient Sunrise® henna has half that level, and it goes on skin rather than being consumed. Mercury content in fruits and vegetables considered safe for eating ranges from 0.1 ppm to 0.02 ppm,³⁹ Ancient Sunrise® henna is in that range. The average cadmium level in vegetables considered safe for consumption, 0.01 to 0.22 ppm with a mean of 0.04 ppm, is at about the same as the level in Ancient Sunrise® henna.⁴⁰ In comparison, one cigarette contains about 0.5 - 2 µg of cadmium and about 10% of the cadmium content is inhaled when the cigarette is smoked.⁴¹

Additional phytochemistry of henna

Plants are composed of elements, substances such as hydrogen, carbon, and nitrogen, and combinations of elements: molecules. The most common molecule in plants, and of course, henna, is dihydrogen monoxide, H₂O, more commonly known as water. The main chemical components of plants, including henna, are water, carbon compounds, soluble and polymeric sugars, organic acids, and mineral substances, all of which have rather long scientific names for scientific specificity. Phytochemicals are naturally-occurring parts of the henna plant, just as potato starch, ((C₆H₁₀O₅)_n, a polysaccharide (q.v.) comprising glucose monomers joined in α 1,4 linkages)⁴² naturally occurs in the tubers of the potato plant, and is not an additive.

The phytochemistry of henna (the molecules which naturally occur in the henna plant) has been meticulously studied, isolating flavonoids, tannins, naphthalenes, xanthenes, lignans, and other compounds. There are smaller amounts of terpenes. The complete details of the phytochemistry of henna and relevant research papers on the following list of phytochemicals isolated thus far (as of 2014) from the henna plant can be found in "Lawsonia inermis L. (henna): Ethnobotanical, phytochemical and pharmacological aspects."⁴³

³⁸ SILLIKER, Inc. Southern California Laboratory Merieux NutriSciences (2017) report on JAR19917TD2_1:070517hRUT Client: TapDancing Lizard, LLC

³⁹ Gerdes, R.A., Hardcastle, J.E., Stabenow, K.T. (1974) "Mercury content of fresh fruits and vegetables" *Chemosphere* Volume 3, Issue 1, February, Pages 13-18

⁴⁰ Thomas, B., Roughan, J. A., Watters, E. D. (1972) "Lead and cadmium content of some vegetable foodstuffs" *Journal of the Science of Food and Agriculture*, Volume 23, Issue 12, December 1972, Pages 1493-1498

⁴¹ World Health Organisation (WHO) (1992). Environmental Health Criteria 134 - Cadmium International Programme on Chemical Safety (IPCS) Monograph.

⁴² IUPAC Name: 5-[3,4-dihydroxy-6-(hydroxymethyl)-5-methoxyoxan-2-yl]oxy-6-[[3,4-dihydroxy-6-(hydroxymethyl)-5-methoxyoxan-2-yl]oxymethyl]-2-[4,5-dihydroxy-2-(hydroxymethyl)-6-methyloxan-3-yl]oxyoxane-3,4-diol

Henna leaves and other parts of the plant also contain tannins; tannic acid is one of the main constituents of the plant.⁴⁴

Alkylphenones have been isolated and identified in henna leaves: Lalioid (2,3,4,6-tetrahydroxyacetophenone-2-O- β -D-glucopyranoside), lawsoniaside A (1-butanoyl-3,5-dimethylphlorogluciny-6-O- β -D-glucopyranoside), and 2,4,6-trihydroxyacetophenone-2-O- β -D-glucopyranoside.⁴⁵

Lawsoniaside B (3-(4-O- α -D-glucopyranosyl-3,5-dimethoxy) phenyl-2E-propenol) and syringinosin di- β -D-glucopyranoside have been isolated from henna leaves.⁴⁶ Other compounds have been isolated from stems and leaves: obtusafuran derivative, lawsonicin (2,3-dihydro-5-hydroxy-3-(hydroxymethyl)-2-[4-((3-hydroxypropyl)-3-methoxy)phenyl]-6-methoxy-1-benzofuran), p-coumaric acid and gallic acid.⁴⁷

Xanthenes were isolated in an analysis of sample material taken from the whole plant: of 1,3-dihydroxy-6,7-dimethoxyxanthone (also referred to as laxanthone-I) (47); 1-hydroxy-3,6-diacetoxy-7-methoxyxanthone (laxanthone-II) (48) and 1-hydroxy-3,7-dimethoxy-6-acetoxyxanthone (laxanthone-III).⁴⁸

Henna flower essential oil contains α -ionone and β -ionone, 2-phenylethanol, and benzyl alcohol, together with other alcohols and aldehydes. Leaf volatiles include methyl- α -D-glucopyranoside, 2-hydroxy-1,4-naphthalenedione, ethyl hexadecanoate, (E)-methyl cinnamate, isocaryophyllene and methyl linolenate. Other terpenes isolated from henna that are included in henna leaf essential oil are 1,8-cineole, α -pinene and p-cymene bisabolene, eugenol, hexadecanoic acid, phytol, α -terpineol and etherphenylvinyl. Non-volatile terpenoids were also isolated from the leaves: Lupeol botulin, betulinic acid, 30-norlupan-3 β -ol-20-one.⁴⁹

⁴³ Semwala, R. B., Semwala, D. K., Combrinck, S., Cartwright-Jones, C., Viljoen, A. (2014) "Lawsonia inermis L. (henna): Ethnobotanical, phytochemical and pharmacological aspects." *Journal of Ethnopharmacology*, June 2014

⁴⁴ Ibid p. 7

⁴⁵ Ibid p. 9.

⁴⁶ Ibid p. 9.

⁴⁷ Ibid p. 6 - 9

⁴⁸ Ibid p. 7

⁴⁹ Ibid p. 10

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Part 2: The Phytochemistry of Henna, Lawsonia inermis L. [Lythraceae]

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