Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(4), 1861-1868 | ISSN:2251-6727

Comparative Phytochemical Analysis of Henna (Lawsonia Inermis) in Suspected Counterfeit Marketed Samples

Nishma Sherin .KV[1], Bency Baby .T[*1], Shibly Rahman .P[1], Safwan .M[1], Rinshad .P[1], Ismail .K[1]

[1]Department of Pharmacognosy, Al Shifa College of Pharmacy, Affiliated to Kerala University of Health Sciences, Thrissur, Kerala, 680596, India

*** Corresponding Author: - Bency Baby .T,**

(Received: 14 April 2024 Revised: 1 May 2024 Accepted: 18 June 2024)

KEYWORDS

Henna, *Lawsonia inermis*, Cosmetic, Adulteration, Quality assessment, Phytochemical analysis, HPTLC.

ABSTRACT:

Henna (*Lawsonia inermis* L., Family- Lythraceae) is widely used in cosmetic applications, but the market is inundated with counterfeit products lacking quality assessment, posing risks to consumers. This study aimed to evaluate the quality of marketed henna formulations compared to authentic samples using organoleptic characters, physicochemical properties, preliminary phytochemical analysis, and High-Performance Thin-Layer Chromatography (HPTLC). Phytochemical screening revealed a diverse chemical profile, with significant variations in alkaloids, saponins, and steroids among samples. The physicochemical evaluation highlighted elevated ash values in marketed samples, suggesting potential adulteration. HPTLC fingerprinting identified unique phytoconstituents, aiding in the differentiation between authentic and counterfeit samples. This comprehensive analysis underscores the need for stringent quality control measures to ensure consumer safety and maintain the integrity of herbal products in the cosmetic industry.

1. INTRODUCTION

Henna (*Lawsonia inermis* L., Family- Lythraceae) has been a staple in cosmetic practices over centuries, renowned for its natural dyeing properties used in body art, hair dyeing, and various cultural rituals. Henna, historically revered as a cultural emblem, has been extensively utilized in the Arabian Peninsula, Indian Subcontinent, Southeast Asia, and parts of North Africa [1]. The dye molecule lawsone, predominantly concentrated in henna leaves, imparts significant value to skin, hair, and textile dyeing practices, as well as in Ayurveda, where it is recognized as Mehndi [2]. Crude extracts of henna and its ingredients have been reported to exhibit various biological activities, including antibacterial, antioxidant, anti-inflammatory, and anticancer properties [3]. Pure henna is generally considered safe, with minimal reports of allergic reactions despite its widespread use [4]. Lawsone derivatives have shown efficacy against various pathogens, indicating their potential as anti-parasitic agents [5]. Lawsone is the principal active ingredient in henna, making it a versatile and valuable natural resource [6].

However, the henna market is rife with counterfeit and adulterated products, which is evident from undesired skin reactions to these products reported occasionally in certain parts of the world, raising quality and safety concerns for consumers. To address this issue, a standardized method for comparative phytochemical analysis of suspected counterfeit marketed henna samples against authentic ones is imperative.

Drawing from previous studies on purity analysis of pharmaceuticals, [7] the occurrence of counterfeit plant drugs [8], and the potential nutraceutical properties of plant extracts [9], this research aims to evaluate the chemical composition of henna samples. By utilizing organoleptic characteristics, physicochemical properties, preliminary phytochemical analysis, and High-Performance Thin-Layer Chromatography (HPTLC), this study sought to discern any discrepancies in the phytochemical profiles of marketed henna samples compared to genuine ones.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(4), 1861-1868 | ISSN:2251-6727

Previous research on the estimation of lawsone in *Lawsonia inermis* [10], and phytochemical analysis of henna leaves [11] provides the foundational framework for understanding the chemical constituents and potential therapeutic properties of henna.

In this context, our study aimed to highlight the differences in constituents between marketed henna cones and authentic samples, utilizing physicochemical, phytochemical, and chromatographic evaluations. By scrutinizing popular henna cones in the Kerala market (coded as H1, H2, H3, and H4), we aim to ensure consumer safety and detect potential adulteration. The study incorporates High-Performance Thin-Layer Chromatography (HPTLC) for a comprehensive analysis. The appendix provides the correlation between codes and specific brands for transparency and completeness.

This study builds upon existing knowledge to provide insights into the authenticity and quality of henna products available in the market, with the aim of enhancing consumer awareness and safety in the cosmetic industry.

2. OBJECTIVES

- Identify suspected marketed counterfeit samples of henna.
- To compare the phytoconstituents present in the various samples.
- To develop an HPTLC fingerprint for evaluating the qualitative status of henna.

3. METHODS

Procurement and authentication of plant materials

Fresh leaves of *Lawsonia inermis* were collected from the medicinal garden of the Al Shifa College of Pharmacy, Perinthalmanna, and authenticated by the Pharmacognosy Division at the Centre for Medicinal Plant Research, Arya Vaidya Sala Kottakkal [Reference No: CMPR/AIF/PHG/317]. Leaves were washed, shade-dried, powdered, and used for extraction.

Collection of Marketed Samples

Four henna samples from brands Big-B, Red Chilly, Wafa, and Singh were obtained from the local market in the Perinthalmanna region, Kerala, India. These samples represent commonly available products in the local market.

Chemicals:

HPTLC grade solvents (methanol and water) were purchased from Merck Ltd., Mumbai, India, and analytical grade chemicals were purchased from Nice Chemicals Pvt. Ltd. Ernakulam, India, was used.

Preliminary Phytochemical Screening

Standard methods have been employed for screening various phytochemical constituents, including carbohydrates, proteins, amino acids, alkaloids, saponins, steroids, flavonoids, tannins, phenolic compounds, glycosides, and lipids. [12]

Physicochemical Evaluation

The moisture content, ash value, water-soluble extractive value, and alcohol-soluble extractive value of the henna samples were determined following standard procedures. [13]

High-performance thin-layer chromatography (HPTLC) fingerprinting

Fresh *Lawsonia inermis* leaves were sun-dried, powdered, and macerated in alcohol for 24 h with intermittent shaking. The resulting extract was then filtered and evaporated under reduced pressure. An ethanolic extract with a concentration of 1 mg/ml was prepared and filtered for HPTLC fingerprinting. Four market samples of henna along with a standard authentic sample, were applied to pre-coated TLC plates using the solvent system (Toluene: ethyl acetate: methanol, 7:3:1).

The HPTLC analysis was carried out using CAMAG equipment, which included a Linomat 4 sample applicator, a micro syringe (100 µl, Hamilton), a reprostar3, a Twin trough chamber, a Dip tank, and Win Cat software (Version 1.3.3). A pre-coated silica gel plate (60 F 254 from Merck, 10 × 10 cm) and an optimized solvent system (toluene: ethyl acetate: methanol, 7:3:1) were used. Samples and standards were applied as bands on the plates in volumes of 5 µL, and detection was performed under UV light at 254 and 366 nm. The Rf values and peak areas of the different phytochemicals were recorded for subsequent analyses. [14]

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(4), 1861-1868 | ISSN:2251-6727

4. RESULTS AND DISCUSSION

Determination of Phytochemical Parameters

Phytochemical analysis of *Lawsonia inermis* samples (H0, H1, H2, H3, and H4) revealed a diverse chemical profile. Carbohydrate tests showed positive results for H0, H2, and H4, whereas proteins and amino acids yielded negative outcomes across all samples. The alkaloid tests displayed positive results for H0, H2, H3, and H4, with H1 showing a negative outcome. Saponin assessment revealed positivity for H0, H1, and H3. Steroid detection revealed H1 positivity, whereas flavonoid analysis showed variable results. Tannin and phenolic tests were negative for all samples, and glycoside testing yielded negative outcomes, with no lipid detected.

These findings underscore the complex phytochemical composition of *Lawsonia inermis* and the importance of stringent quality control measures to combat counterfeit herbal products in the market [15-16].

Table 1: Phytochemical parameters

Sl. No	CONSTITUENTS	TEST	RESULTS				
			Ho	H1	H2	H3	H4
1	Carbohydrate	Molisch's test	+	-	+	-	+
		Benedict's test	+	-	+	-	+
		Fehling's test	+	-	+	-	+
2	Proteins and amino acids	Biuret test	-	-	-	-	-
		Millon's test	-	-	-	-	-
3	Alkaloids	Mayer's test	+	-	+	+	+
		Dragendroff' test	+	-	+	+	+
4	Saponins	Foam test	+	+	-	+	-
5	Steroids	Salkowski test	-	+	-	-	-
		Libermann's test	-	+	-	-	-
6	Flavonoids	Shinoda test	-	+	+	+	-
		Lead acetate test	-	+	+	+	-
7	Tannins and Phenolics	Ferric chloride test	-	-	-	-	-
		Lead acetate test	-	-	-	-	-
		Gelatine test	-	-	-	-	-
8	Glycosides	Legal's test	-	-	-	-	-
		Keller-Killani test	-	-	-	-	-
		Conc.H_2SO_4 test	-	-	-	-	-
9	Lipids		-	-	-	-	-

Determination of Physicochemical Parameters

Physicochemical parameters were used to assess the quality and purity of the samples. For the authentic henna sample (H0), the total ash value was 3.0% w/w, accompanied by a minimal acid-insoluble ash content of 1.34% w/w, signifying the low presence of inorganic components. In contrast, the marketed samples (H1, H2, H3) displayed diverse total ash values (13.7% w/w, 8.45% w/w, 5.92% w/w) and acid-insoluble ash values (1.02% w/w, 2.23% w/w, 0.53% w/w), indicating significant variations in inorganic content. The extractive values further enriched our understanding, with consistently higher water-soluble extractive values across all plant materials than their alcohol-soluble counterparts. Specifically, the water-soluble extractive values were 15% for H0, 22% for H1, 18% for H2, 25% for H3, and 19% for H4. The alcohol-soluble extractive values were 12% for H0, 17% for H1, 14% for H2, 20% for H3, and 16% for H4. Overall, physicochemical parameters provided valuable insights into the quality and purity of *Lawsonia inermis* samples. The authentic henna sample (H0) exhibited low total ash and acid-insoluble ash values, indicating a low presence of inorganic components. In contrast, the marketed samples (H1, H2, and H3) displayed varying total ash values, reflecting significant differences in inorganic content compared with the reference sample (H0). The consistently higher water-soluble extractive values across all samples compared to their alcohol-soluble counterparts further emphasize the variations in their compositions. These results underscore the paramount importance of rigorously evaluating a comprehensive range of physicochemical parameters to effectively ascertain the authenticity and quality of henna products, particularly in the critical task of identifying potentially counterfeit or adulterated samples. This thorough

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(4), 1861-1868 | ISSN:2251-6727

assessment is a crucial step in ensuring consumer safety and maintaining the integrity of herbal products. [17-18]

Table 2. Physiochemical parameters

SL NOS	PARAMETER	RESULT (% W/W)				
		Ho	H1	H2	H3	H4
1	Total ash	3.0	21.35	5.82	40.59	4.62
2	Acid insoluble ash	0.704	18.929	3.436	38.269	2.464
3	Moisture content	89.33	10.66	51.33	18	38.66
4	Water-Soluble Extractive Value	15	22	18	25	19
5	Alcohol-Soluble Extractive Value	12	17	14	20	16

High-Performance Thin-Layer Chromatography (HPTLC)

The High-Performance Thin-Layer Chromatography (HPTLC) fingerprinting analysis conducted on *Lawsonia inermis* standard [H0] and suspected counterfeit henna samples [H1, H2, H3, H4] revealed significant insights into the phytochemical composition of these samples. The results in Tables 3–7 show distinct peaks with varying maximum Rf values, maximum heights, and area percentages at 254 and 366 nm at UV wavelengths for each sample. Comparative analysis of the HPTLC fingerprinting profiles between the standard [H0] and suspected counterfeit samples [H1-H4] revealed notable differences in peak intensities, Rf values, and area percentages. For instance, in the sample [H1], the peaks at Rf values of 0.02 and 0.24 exhibited significant differences in maximum heights and area percentages compared to the standard [H0]. Similarly, sample [H2] displayed variations in peak characteristics at Rf values of 0.01, 0.19, and 0.36. In sample [H3], distinct Rf values were identified at 254 nm (0.02, 0.22, 0.34, 0.69, and 0.95) and 366 nm (0.21 and 0.95), providing valuable insights into the migration behavior of compounds within the sample at different UV wavelengths. Sample [H4] also exhibited distinct peaks at various Rf values, indicating differences in the phytochemical composition compared to the standard [H0]. The results of this study revealed the utility of HPTLC fingerprinting in the analysis of phytochemical profiles of plant extracts [10,19]. The unique bands observed in the suspected counterfeit samples highlight the potential of HPTLC fingerprinting to differentiate authentic and adulterated henna products. The presence of specific bands and variations in peak areas among the samples underscores the importance of HPTLC as a reliable technique for quality assessment and authenticity verification of herbal products [20-21]. HPTLC fingerprinting analysis of henna samples in suspected counterfeit marketed samples demonstrated its efficacy in identifying unique phytoconstituents and discerning variations among samples. This study contributes to the growing body of knowledge on the application of HPTLC in the quality assessment and authenticity verification of herbal products, particularly in the context of combating adulterated products in the market.

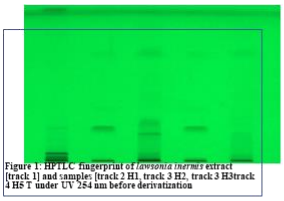
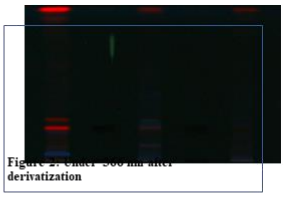


Figure 1: HPTLC fingerprint of *lawsonia inermis* extract [track 1] and samples [track 2 H1, track 3 H2, track 3 H3track 4 H5 T under UV 254 nm before derivatization



derivatization

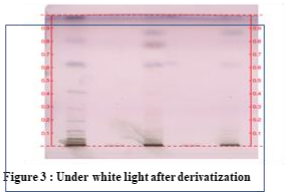


Figure 3 : Under white light after derivatization

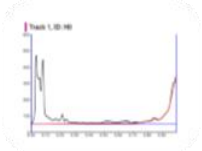


Fig 4: Single track scan chromatograms of H0 under 254 nm

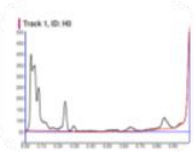


Fig 5: Single track scan chromatograms of H0 under 366 nm

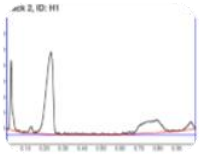


Fig 6 : Single track scan chromatograms of H1 under 254 nm

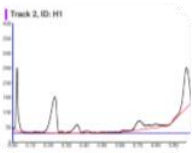


Fig 7 : Single track scan chromatograms of H1 under 366 nm

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(4), 1861-1868 | ISSN:2251-6727

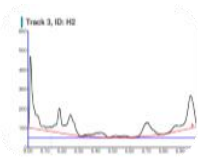


Fig 8: Single track scan chromatograms of H2 under 254 nm

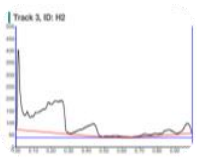


Fig 9: Single track scan chromatograms of H2 under 366 nm

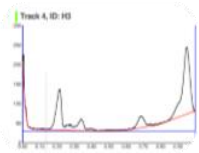


Fig 10: Single track scan chromatograms of H3 under 254 nm

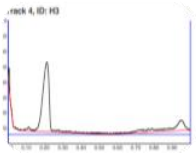


Fig 11: Single track scan chromatograms of H3 under 366

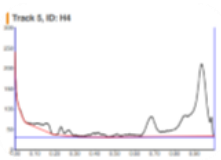


Fig 12: Single track scan chromatograms of H4 under 254 nm

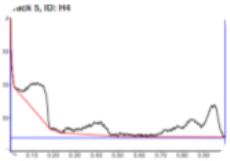


Fig 13: Single track scan chromatograms of H4 under 366 nm

Table 3: Details of HPTLC fingerprinting *of Lawsonia inermis* standard [H0] at different visibilities.

Peak	Max Rf at 254 nm	Max height at 254 nm	Area % at 254 nm	Max Rf At 366 nm	Max height at 366 nm	Area % at 366 nm
1	0.03	421.5	39.19	0.04	345.2	20.81
2	0.08	395.3	43.08	0.06	294.9	29.59
3	0.17	32.3	3.08	0.08	195.2	16.89
4	0.22	57.6	6.02	0.12	37.8	4.46
5	0.24	29.2	3.05	0.24	134.3	13.05
6	0.52	15.4	2.25	0.29	24.9	2.28
7	0.67	15.3	0.55	0.64	19.6	3.40
8	0.85	15.6	1.93	0.84	51.4	9.51

Table 4: Details of HPTLC fingerprinting of Henna sample [H1] at different visibilities.

Peak	Max Rf at 254 nm	Max height at 254 nm	Area % at 254 nm	Max Rf At 366 nm	Max height at 366 nm	Area % at 366 nm
1	0.02	212.1	27.80	0.02	220.5	40.68
2	0.24	122.4	48.61	0.13	20.8	2.06
3	0.36	29	11.27	0.23	259.1	58.49
4	0.70	30.2	12.31	0.75	37.1	10.59
5.	-	-	-	0.80	40.2	10.20
6.	-	-	-	0.97	23.5	3.97

Table 5: Details of HPTLC fingerprinting of Henna sample [H2] at different visibilities.

Peak	Max Rf at 254 nm	Max height at 254 nm	Area % at 254 nm	Max Rf at 366 nm	Max height at 366 nm	Area % at 366 nm
1	0.01	369.4	37.14	0.01	331.3	20.74
2	0.19	126.3	15.69	0.07	80.0	6.96
3	0.25	102.7	18.06	0.11	79.1	4.72
4	0.36	30.0	1.52	0.19	125.9	18.16
5	0.41	22.0	2.81	0.23	133.6	13.01
6	0.43	23.7	2.98	0.26	138.1	15.13
7	0.70	69.5	16.71	0.44	53.0	12.39
8	0.87	31.0	5.09	0.86	21.6	3.58

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(4), 1861-1868 | ISSN:2251-6727

Table 6: Details of HPTLC fingerprinting of Henna sample [H3] at different visibilities.

Peak	Max Rf at 254 nm	Max height at 254 nm	Area % at 254 nm	Max Rf At 366 nm	Max height at 366 nm	Area % at 366 nm
1	0.02	12.9	0.56	0.21	226.2	87.22
2.	0.22	104.8	54.63	0.95	33.6	12.78
3	0.34	29.7	7.17	-	-	-
4	0.69	29.0	8.45	-	-	-
5	0.95	171.7	59.19	-	-	-

Table 7: Details of HPTLC fingerprinting of Henna sample [H4] for different visibilities.

Peak	Max Rf at 254 nm	Max height at 254 nm	Area % at 254 nm	Max Rf At 366 nm	Max height at 366 nm	Area % at 366 nm
1	0.16	21.1	6.63	0.16	52.8	47.23
2	0.23	21.3	2.43	0.42	20.5	6.28
3	0.27	14.4	2.36	0.84	26.3	14.10
4	0.69	48.7	14.51	0.95	48.4	32.39
5	0.86	51.2	13.51	-	-	-
6	0.94	176.82	57.86	-	-	-
7	0.99	45.9	2.71	-	-	-

The assessment of counterfeit cosmetic formulations containing *Lawsonia inermis* (henna) through a comprehensive analysis of physicochemical standards, phytochemical profiles, and chromatographic evaluations revealed significant deviations from authentic henna samples (H1). This study identified potential adulteration in the raw material based on elevated ash values in all samples compared to the standard. The HPTLC fingerprinting method proved to be a reliable tool for rapid screening and plant identification, showing notable variations in Rf values among brands and authentic henna samples. However, the fact that henna cones for application on palms have additional ingredients such as tea, coffee, sugar, etc to improve the texture and stability of formulation invokes the need to consider these factors while preparing the standard for comparison against commercial samples. Further, the need for pre-written standards for ingredients that can be added to commercial preparations of henna, their shelf lives, storage conditions, etc must be addressed through advanced and well-designed studies. Although further investigations are warranted, the findings of this study provide valuable insights into the need to ensure the authenticity and safety of *Lawsonia inermis* in cosmetic formulations. Continuous monitoring programs are recommended to address potential adulteration and to safeguard consumers.

ACKNOWLEDGMENT

We would like to express our sincere gratitude to Al Shifa College of Pharmacy for providing the resources and facilities necessary to conduct this research and to the Phytochemistry Division, Centre for Medicinal Plants Research (CPMR) Arya Vaidya Sala, Kottakkal for facilitating the HPTLC analysis.

REFERENCES

1. Barani, M.; Mirzaei, M.; Torkzadeh-Mahani, M.; Nematollahi, M. H. Lawsone-Loaded Niosome and Its Antitumor Activity in MCF-7 Breast Cancer Cell Line: A Nano-Herbal Treatment for Cancer. *Daru Journal of Pharmaceutical Sciences* **2018**, *26* (1), 11–17. https://doi.org/10.1007/s40199-018-0207-3.
2. Sousa, R. M.; Rodrigues, F. V.; Medrado, B. L.; Oliveira, L. M.; Pereira, Í. G.; Amantino, C. F.; Goto, P. L.; Blanzat, M.; Primo, F. L.; Tedesco, A. C.; Siqueira-Moura, M. P. Development and In Vitro Cytotoxicity Assessment of Nanoemulsified Lawsone. *Chem. Pap.* **2022**, *76* (8), 5043–5050. https://doi.org/10.1007/s11696-022-02242-9

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(4), 1861-1868 | ISSN:2251-6727

3. Almeida, P. J., Borrego, L., Pulido-Melián, E., & González-Díaz, O. Quantification of p-Phenylenediamine and 2-Hydroxy-1,4-Naphthoquinone in Henna Tattoos. *Contact Dermatitis* **2012**, *66* (1), 33-37. https://doi.org/10.1111/j.1600-0536.2011.01992.x
4. Al Nasr, I.; Jentzsch, J.; Winter, I.; Schobert, R.; Ersfeld, K.; Koko, W. S.; Mujawah, A. A.; Khan, T. A.; Biersack, B. Antiparasitic Activities of New Lawsone Mannich Bases. *Arch. Pharm. (Weinheim)* **2019**, *552* (11), 1900128. https://doi.org/10.1002/ardp.201900128
5. Jordão AK, Vargas MD, Pinto AC, da Silva FD, Ferreira VF. Lawsone in organic synthesis. *RSC Adv.* **2015**, 5 (83),67909-67943. https://doi.org/10.1039/C5RA12785H
6. Pradhan, R.; Dandawate, P.; Vyas, A.; Padhye, S.; Biersack, B.; Schobert, R.; Ahmad, A.; Sarkar, F. H. From Body Art to Anticancer Activities: Perspectives on Medicinal Properties of Henna. Curr. Drug Targets **2012**, 13 (14), 1777–1798. https://doi.org/10.2174/138945012804545588
7. Fakayode SO. Purity analysis of the pharmaceuticals naproxen and propranolol: A guided-inquiry laboratory experiment in the analytical chemistry laboratory. Journal of Chemical Education. **2015** Jan 13;92(1):157- . https://doi.org/10.1021/ed500282h.
8. da Silva, A. R.; de Lima Silva, C.; do Nascimento Júnior, W.; de Sousa Alves, A. K.; Santana, L. S.; Gonçalves, J. N.; Soares, F. P.; de Matos Dourado, R. C. Estudo morfoanatômico e fitoquímico de amostras de sementes comercializadas como Noz-da-Índia (Aleurites moluccana). Research, Society and Development **2020**, 9 (7), e07973518. https://doi.org/10.33448/rsd-v9i7.3518
9. Amat-ur-Rasool, H.; Symes, F.; Tooth, D.; Schaffert, L. N.; Elmorsy, E.; Ahmed, M.; Hasnain, S.; Carter, W. G. Potential Nutraceutical Properties of Leaves from Several Commonly Cultivated Plants. Biomolecules **2020**, 10 (11), 1556. https://doi.org/10.3390/biom10111556
10. Garg, R. K.; Tripathi, R.; Batav, N.; Singh, R. K. Phytochemical High Performance Thin Layer Chromatography Based Estimation of Lawsone in Lawsonia inermis (Henna) Obtained from Two Natural Habitats and Dye Products Collected from Local Market. Med Aromat Plants (Los Angels) **2017**, 6 (3), 2167-0412. https://doi.org/10.4172/2167-0412.1000290.
11. Wagini, N. H.; Soliman, A. S.; Abbas, M. S.; Hanafy, Y. A.; Badawy, E. S. Phytochemical Analysis of Nigerian and Egyptian Henna (Lawsonia inermis L.) The leaves were analyzed by TLC, FTIR, and GC-MS. Plant **2014**, 2 (3), 27-32. https://doi.org/10.11648/j.plant.20140203.11.
12. Dahake, P. R.; Kamble, S. I. Study on Antimicrobial Potential and Preliminary Phytochemical Screening of Lawsonia inermis Linn. *Int. J. Pharm. Sci. Res.* **2015**, 6 (8), 3344-3350.https://doi.org/10.13040/IJPSR.0975-8232.6(8).3344-50
13. Zahara, F.; Harahap, U.; Haro, G. I. Physicochemical Properties, Phytochemical Screening, and Antihyperglycemic Activity of Inai (*Lawsonia inermis* L.) Leaves Active Fraction on Streptozotocin-Induced Diabetic Mice. *Asian J. Pharm. Clin. Res.* **2018**, *11* (5), 68–71. http://dx.doi.org/10.22159/ajpcr.2018.v11i5.23504
14. Gallo, F. R.; Multari, G.; Giambenedetti, M.; Federici, E. Chemical Fingerprinting of Lawsonia inermis L. Using HPLC, HPTLC, and Densitometry. Phytochem. Anal. **2008**, 19(6), 550-559. https://doi.org/10.1002/pca.1084
15. Al-Snafi, A. E. A Review on Lawsonia inermis: A Potential Medicinal Plant. *Int. J. Curr. Pharm. Res.* **2019**, 11 (5), 1-3. http://dx.doi.org/10.22159/ijcpr.2019v11i5.35695
16. Gull, I.; Sohail, M.; Aslam, M. S.; Athar, M. A. Phytochemical, Toxicological and Antimicrobial Evaluation of Lawsonia inermis Extracts Against Clinical Isolates of Pathogenic Bacteria. *Ann. Clin. Microbiol. Antimicrob.* **2013**, *12*, 1-6. https://doi.org/10.1186/1476-0711-12-36
17. Charoensup, R.; Duangyod, T.; Palanuvej, C.; Ruangrungsi, N. Pharmacognostic Specifications and Lawsone Content of Lawsonia inermis Leaves. *Pharmacogn. Res.* **2017**, *9* (1), 60. https://doi.org/10.4103/0974-8490.199775.
18. Baskaran, K.; Suruthi, B. Hepatoprotective Activity of Ethanolic Seed Extract of Lawsonia inermis Against Paracetamol-Induced Liver Damage in Rats. *Sch. J. App. Med. Sci.* **2016**, *4* (7C), 2488-2491. https://doi.org/10.36347/sjams.2016.v04i07.038.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(4), 1861-1868 | ISSN:2251-6727

19. Tistaert, C.; Dejaegher, B.; Vander Heyden, Y. Chromatographic Separation Techniques and Data Handling Methods for Herbal Fingerprints: A Review. *Anal. Chim. Acta* **2011**, *690* (2), 148-161. https://doi.org/10.1016/j.aca.2011.02.023.
20. Garg, M.; Dwivedi, N. Physicochemical and Phytochemical Studies on Sphaeranthus indicus Linn. by HPTLC Fingerprinting. *J. Drug Deliv. Ther.* **2021**, *11* (2), 100-107. https://doi.org/10.22270/jddt.v11i2.4750.
21. Shanaida, M.; Jasicka-Misiak, I.; Makowicz, E.; Stanek, N.; Shanaida, V.; Wieczorek, P.P. Development of a High-Performance Thin Layer Chromatography Method for Identification of Phenolic Compounds and Quantification of Rosmarinic Acid Content in Some Species of the Lamiaceae Family. *J. Pharm. Bioallied Sci.* **2020**, *12* (2), 139-145. https://doi.org/10.4103/jpbs.JPBS_322_19.