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Scientific Basis for the Innovative Uses of Henna (*Lawsonia inermis* L.) mentioned by Unani Scholars in different ailments

Jamal Akhtar[1*], Fouzia Bashir[1] and Salma Bi[2]

[1]Central Council for Research in Unani Medicine, New Delhi, India.
[2]Jamia Tibbiya Deoband- Saharanpur, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The Unani Medicine is a comprehensive medical system, which meticulously deals with the various states of health and disease. It provides promotive, preventive, curative and rehabilitative healthcare. The fundamentals, diagnosis and treatment modalities of the system are based on scientific principles and holistic concepts of health and healing. The history of Unani Medicine is traced back to ancient Egypt and Babylon, but Hippocrates (460–370 BC) is the father of Unani Medicine who set the ground for Medicine to develop it as a systematic science. Other scholars like Galen (129-200 AD); *Rabban Ṭabarī* (775-890 AD), al Rāzī (865-925 AD) and Ibn Sīnā (980-1037 AD) developed Unani Medicine to great heights. The system is enriched with detailed therapeutic uses of plant, mineral and animal origin drugs. Ibn Baytār's classical pharmacopoeia describes 1400 medicinal plants and minerals, while the largest Indian compendium by *Muḥammad Najm al Ghanī* published in 1930, describes 2500 natural products. Unani scholars retain its traditional strength successfully and also benefitting from contemporary scientific development over the years. The system fully appropriated the paradigm of validation of existing knowledge on modern scientific parameters as well as new action of existing drugs was also

**Corresponding author: E-mail: jamal7862@yahoo.co.in;*

discovered. Henna (*Lawsonia inermis* L.) is a commonly used medicinal plant of Unani Medicine which has Blood Purifier, Abortifacient, Analgesic, Astringent, Brain Tonic, Diuretic properties and used in the treatment of different diseases, e.g. *Jarb-o-Hikka* (Scabies), *Juzaam* (Leprosy), *Bars* (Leukoderma), *Iltehaab-e-Mafasil* (Arthritis), *Dunbal* (Boils) and *Zarba-o-Saqta* (bruises), *Su'aal* (Bronchitis), *Harq-o-Salq* (Burn and Scald), *Humrah Dimaaghia* (Erysipelas), *Hasbah* (Measles) and *Judree* (Small Pox) and *Suda'* (Headache). This paper is a view to present a snapshot of the scientific aspects and the relevance of Henna (*Lawsonia inermis* L.) mentioned by Unani Physicians in the contemporary and emerging health scenario.

Keywords: Henna; medicinal uses; antifungal activity; antipyretic; anti-inflammatory.

1. INTRODUCTION

The Unani System of Medicine has history which is traced back to ancient Egypt and Babylon. Egyptian physicians e.g. Imhotep (2800 BC) and Amenhotep (1550 BC) had adopted the use of medicinal plants for different ailments. Babylonians, who used urine sample as a diagnostic tool, also occupied an important place in the history of Unani Medicine. During the period of Asclepius (1200 BC), the Greeks worked and developed different art of medicine which was based on medical knowledge of Egyptians and Babylonians. Hippocrates (460–370 BC) was the dominating figure of the classical period of Greek medical history who set the ground for Medicine to develop it as a systematic science. Other scholars like Galen (129-200 AD); *Rabban Ṭabarī* (775-890 AD), al Rāzī (865-925 AD) and Ibn Sīnā (980-1037 AD) developed Unani Medicine to great heights. The system was introduced in India during the eighth century AD. The system is enriched with detailed therapeutic uses of plant, mineral and animal origin drugs. Ibn Baytār's classical pharmacopoeia describes 1400 medicinal plants and minerals, while the largest Indian compendium by Muḥammad Najm al Ghanī published in 1930, describes 2500 natural products. Unani scholars retain its traditional strength successfully and also benefitting from contemporary scientific development over the years. The system fully appropriated the paradigm of validation of existing knowledge on modern scientific parameters as well as new action of existing drugs were also discovered [1]

Henna (*Lawsonia inermis* L.) commonly known as Mehendi is a used Unani medicinal plant. It is a shrub or small tree standing 1.8 to 7.6 m tall. The plant is usually glabrous and multi-branched, with spine-tipped branchlets whereas the leaves grow opposite each other on the stem. They are sub-sessile, elliptical, and lanceolate acuminate (tapering to a long point), and have depressed veins on the dorsal surface. The flowers have four sepals and a 2 mm calyx tube, with 3 mm spread lobes. Petals are ovate, with white or red stamens that are found in pairs on the rim of the calyx tube. The plant has 5 mm long, and erect ovary. The fruits are small, brownish capsules, 4–8 mm (0.16–0.31 in) in diameter, with 32–49 seeds per fruit, and open irregularly into four splits [2,3]

The leaves of the plant are very popular as natural dye to colour hands, fingers, nails and hair. Lawsone is the main constituent of the plant which act as dye molecule. Petioles of the plant shows maximum concentration (0.5-1.5 %) of Lawsone.

1.1 Pharmacological Actions

According to Unani literature, Henna possesses different temperament. According to *Ibn Sina*, Henna has Cold 1^0 and Dry 2^0 temperament. Some physicians say that its flower have only Dry 2^0 temperament. *Baghdadi* says that the whole plant has Cold 1^0 temperament. According to *Ibn Zuhar*, if there is any deformity in nails, application of paste of henna leaves mixed with butter is beneficial. He also mentioned that henna oil mixed with olive oil and Tar is very helpful in Alopecia. Leaves of Henna helps in wound healing. Paste of leaves mixed with white vinegar decreases headache [4-8].

Various actions of the plant mentioned in Unani classical text are presented Table 1.

1.2 Therapeutic Uses of Henna

Henna (*Lawsonia inermis* L.) have a number of therapeutic uses. Some of them described in Unani classical books have been listed Table 2.

Table 1. Pharmacological Actions of *Lawsonia inermis.* L.

S. No.	Pharmacological Actions (Unani Literature)	English terminology	Description of recommendations	References
1.	*Musaffi-e-Dam*	Blood purifier	*Joshanda* (decoction) of leaves is used orally for 15 days.	[9,10]
2.	*Musqit-e- Janeen*	Abortifacient	*Khaisanda* (Decanted water) of leaves of Henna act as abortifacient if taken for 10 days.	[11-13]
3.	*Musakkin-e-Alam*	Analgesic	*Joshanda* (decoction) of Henna flowers can be used orally. Fomentation of affected area with decoction two times a day is beneficial.	[14]
4.	*Mohallil-e-Warm*	Resolvent	*Zimad* (Paste) of the fresh leaves to be used on the affected area.	[9,15,16]
5.	*Daf'e Humma*	Antipyretic	Paste of freshly ground leaves applied on forehead to combat fever.	[8,16]
6.	*Muqawwi-e-Bah*	Aphrodisiac	*Khaisanda* (Decanted water) of Henna leaves acts as aphrodisiac.	[14,15]
7.	*A'fis*	Astringent	It is applied locally as cream that causes the skin or other tissue to tighten.	[5,14]
8.	*Muqawwi-e-Dimagh*	Brain tonic	Powder of henna flowers mixed with honey acts as brain tonic.	[6,14, 17, 11]
9.	*Mujaffif e Qarha*	Desiccant	Paste of leaves is applied on the wounds for healing and gargles with decoction of leaves helps in relieving mouth ulcers.	[14, 18, 8]
10.	*Mudirr-e-Bawl*	Diuretic	Decoction of Henna leaves is taken orally for difficulty in passing urine.	[14, 8, 16, 10]
11.	*Munaffis-e-Balgham*	Expectorant	Decoction of fresh leaves is taken orally to expel out sputum.	[8,13]
12.	*Mudirr-e-Tams*	Emmenogogue	*Joshanda* (decoction) of Henna leaves is taken orally in cases of Amenorrhoea and *Aabzan* (Sitz Bath) with *Joshanda* (decoction) is helpful in uterine diseases.	[8,13]

Table 2. Therapeutic uses of *Lawsonia inermis* L.

S. No.	Therapeutic Uses (Unani Literature)	English Equivalent	Method of application recommended	References
1.	*Jarb-o-Hikka*	Scabies	Applied locally as paste	[12,8,10]
2.	*Juzaam*	Leprosy	Applied locally as paste	[5,6,9,11,14,16,10]
3.	*Bars*	Leukoderma	Applied locally as paste	[12,19,8]
4.	*Iltehaab-e-Mafasil*	Arthritis	Fomentation with decoction	[4,9,12,14,15]
5.	*Dunbal wa Zarba-o-Saqta*	Boils and Bruises	Applied locally as paste and as *Joshanda* (decoction)	[4,5,12,8]
6.	*Su'aal*	Cough	To be taken as *Joshanda* (decoction)	[5,9,17,8]
7.	*Harq-o-Salq*	Burn and Scald	Applied locally as paste	[4,5]
8.	*Usr-e- Bawl*	Dysuria	To be taken as *Joshanda*	[12,14]

S. No.	Therapeutic Uses (Unani Literature)	English Equivalent	Method of application recommended	References
			(decoction)	
9.	*Humrah Dimaghia*	Erysipelas	Applied locally as paste	[5,11]
10.	*Kasrat-e-Lu'ab-e-Dehan*	Excessive salivation	Gargles with decoction	[5]
11.	*Inteshaar-e-Shar*	Falling of Hairs	Topical application of Oil	[9]
12.	*Suda'*	Headache	Applied locally as paste	[4,5,9,12,8,13]
13.	*Waja'-ul-Warik*	Hip Pain	Fomentation with decoction	[4,5,9,8]
14.	*Amraz-e-Kabid*	Liver Diseases	To be taken as *Joshanda* (decoction)	[12,14,16,10]
15.	*Amraz-e-Jild*	Skin Diseases	Applied locally as paste	[16,20]
16.	*Hasbah wa Judree*	Measles and Small Pox	Applied locally as paste	[5,9]
17.	*Amraz-e-Azfaar*	Nails diseases	Applied locally as paste	[5,9,12]
18.	*Izam-e-Tehaal*	Splenomegaly	To be taken as *Joshanda* (decoction)	[12,14,8]
19.	*Qulaa'-e-Dehan*	Stomatitis	Gargles with decoction	[5,6,9,12,14,8,10]
20.	*Khashoonat- e- Halaq*	Sore Throat	Gargles with decoction	[5,6,9,12,14]
21.	*Qurooh-e- Majra-e-Ba'ul*	Urinary Tract Ulcers	To be taken as *Joshanda* (decoction)	[9,21]
22.	*Aatishak*	Syphilis	To be taken as *Joshanda* (decoction)	[8,10]

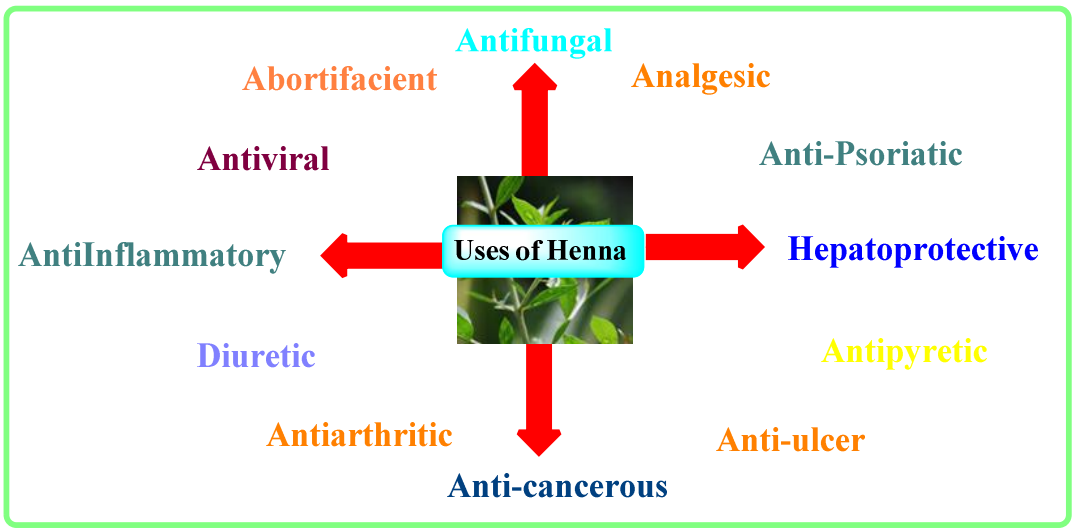

Figure 1: Pharmacological uses of Henna

2. SCIENTIFIC BASIS FOR THE INNOVATIVE USES OF HENNA (*Lawsonia inermis* L.)

Henna is known for its versatile pharmacological activities as antifungal, antibacterial, antiviral, analgesics, anti-inflammatory, anti-ulcer, anti-cancer etc. Here in this section some of the potent pharmacological actions are discussed along with new finding.

2.1 Antifungal Activity

Unani Physicians have described the use of Henna in the treatment of Scabies. Following antifungal studies have been conducted to support the use of Henna in fungal infections-

- The antifungal activity of *Lawsonia inermis* L. (henna) paste was determined by agar diffusion method and it showed the high

antifungal activity against all dermatophytes species (20 to 50 mm inhibition zone). [22]

- The effect of henna on *Candida albicans* was observed in two different methods and it was observed that variation in live *Candida*, between control group and group B (concentration of henna powder was 1%), was significant. Similarly, variations in live *Candida* were significant, when the concentration of powder was 7.5% or 10% in contrast with control group and *p*-values were 0.0001 and 0.001 respectively.[23]
- Extracts of leaves of *Lawsonia inermis* L. (Henna plant) revealed antifungal activity against all yeast strains except *Pichia fabianii* . [24].
- The extract *Lawsonia inermis* L showed broad fungicidal spectrum against 13 ring worm fungi [25].
- Phytochemical analyses of the leaves of *Lawsonia inermis* L. has shown the presence of anthraquinones as major constituents of these leaves and these henna leaves have antibacterial and antifungal activities.[26]
- Omani henna has shown antibacterial activity against a wide spectrum of bacterial strains and *C. albicans* [27].
- *Ethanol*, *methanol* and *aqueous extracts* of leaves of *Lawsonia inermis* L. has shown defensive mechanism against spore germination of *Drechslera oryzae*. In another *in-vitro* study, the ethanolic, ethyl acetate and hexane extracts of *L. inermis* L. were tested against five strains each of *Tinea rubrum* and *Tinea mentagrophytes* and all these extracts have shown significant antidermatophytic properties [28]
- The extract of *L. inermis* L. showed broad fungitoxic spectrum when tested against *Microsporum gypseum* and *Trichophyton mentagrophytes* and13 other dermatophytes [29].
- *Lawsonia inermis* L. leaves extract showed a fungicidal effect against *Trichophyton mentagrophytes* and *Candida albicans* [30].
- Dermatophytes sensitivity toward henna was observed strongly in *Trichophyton mentagrophytes, T. rubrum, T. tonsurans, T. violaceum, T. verrocosum, T. schoenleinii, Epidermophyton floccosum, Microsporum ferrugineum, M. canis* and *sporotrichum schenckii* [31].
- Aqueous extract of *L. inermis* L. leaves has shown significant antifungal activity [32].
- The study confirms the effect of aqueous and methanolic extract of henna using 25 µl of the extracts against *C. albicans* and *Microsporum* [33].
- Lawsone and six extracts of *Lawsonia inermis* L. plant were evaluated for the antifungal activity against filamentous fungi. It was observed that the best yield (8.03%) was obtained with the *ethanol extract*. The lawsone has shown potentially interesting MICs against the strains *Fusarium oxysporum* (12 µg/mL) and *Aspergillus flavus* (50 µg/mL) [34].
- Five medicinal plants, i.e. *Lawsonia inermis*, *Withania somnifera*, *Curcuma longa*, *Cymbopogon citrates* and *Zingiber officinale* were screened against Candida albicans. These plants extracts have shown the potential to control growth of *Candida albicans* [35].
- The antifungal effect of *Lawsonia inermis* L. was compared with clotrimazole on rats. The study showed that vaginal cream prepared from *Lawsonia inermis* L. (henna) can be used for *C. albicans* infections in female rats. It was also observed that 4% henna has similar effect to clotrimazole [36]

2.2 Anti-Inflammatory, Analgesic and Antipyretic Activity

Unani Physicians have described analgesic and anti-inflammatory properties of the plant and they use it in the treatment of arthritis, headache and Hip joint pain. Following studies support the use of *Henna* in the aforesaid diseases-

- *Methanol extract* of *Lawsonia inermis.* flowers has shown anti-inflammatory activity against 5-Lipoxygenase (IC_{50}=49.33mg/L) which may be due to high amounts of total phenolic compounds [37].
- Crude *ethanolic extract* of *L inermis* (0.25-2.0 g/kg) has shown significant and dose-dependent anti-inflammatory, analgesic, and antipyretic activity in rats [38].
- The aqueous extract of *L inermis* leaves exhibited significant ($P<0.05$) anti-inflammatory and analgesic activity whereas alcoholic extract does not show such significant activity [39]
- The *ethanolic extract* of *L inermis* has anti-inflammatory and antioxidant potential [40].

- The *methanolic extract* of *L inermis* in the dose of 500 mg/kg has shown higher analgesic activity against acetic acid-induced pain in mice than the standard drug diclofenac sodium. The extract in the dose of 300 and 500 mg/kg has exhibited sustained inhibition (54.97% and 65.56%) of paw oedema test when compared with standard indomethacin (74.17%). Besides, the plant extract also had significant ($p<0.05$) dose-dependent CNS depressant activity [41]

2.3 Antibacterial Activity

- *Aqueous, methanol* and *chloroform* crude leaves extract of *L. inermis* in different concentrations were investigated for inhibition of the growth of six human pathogenic fungi and four types of bacteria and it was observed that the growth of all pathogens was inhibited to varying degree by increasing the concentration of extract. *Aqueous extract* has shown superior activity followed by methanol and chloroform [26]
- *Methanolic extract* of *Lawsonia inermis* has shown antibacterial activity against *S. aureus* (MTCC 087), *E. coli* (MTCC 729), *K. pneumonia* by Agar well diffusion method. [27]
- *Ethanolic extracts* of 20 selected plant species used by Yemeni traditional healers to treat infectious diseases were screened for their antibacterial activity against both Gram-positive and Gram-negative bacteria, as well as for cytotoxic activity. The ethyl acetate extract of *Lawsonia inermis* L. has shown the most active one against all bacteria in the test system [42]
- Antibacterial activity of *aqueous*, *methanol extracts* of Yemeni henna (*Lawsonia inermis* L.) leaves were tested against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* by using Agar diffusion and Minimum Inhibitory Concentration (MIC). The *methanolic extract* showed more potential antibacterial activity against all the bacterial species than *aqueous extract.* The maximum activity was observed in *methanolic extract* against *Staphylococcus aureus* and minimum activity was observed in aqueous extract against *Escherichia coli* [43].
- *Ethanolic extract* of the leaves of Henna were studied for antimicrobial property using Agar well diffusion method and it was found to inhibit the growth pattern of *A. niger, F. oxysporun*, *Streptococcus* sp and *S. aureus* [44].
- *Aqueous extract* of henna leaf showed antibacterial activity against *Bacillus cereus*, *B. anthracis*, *Staphylococcus aureus*, *E. coli*, *Proteus vulgaris*, *Erwinia carotovora*, *X. canpestris* and *A. tumefaciens* [45].
- *Ethanolic extract* of *Lawsonia inermis* L. has shown antibacterial properties over a wide range of disease-causing gram positive bacteria (*Bacillus megaterium, Bacillus subtilis, Bacillus fusiformis, Streptococcus pyogenes, Streptococcus faecalis, Staphyloccus aureus, Streptococcus pneumonia,*) as well as gram negative bacteria (*Salmonella typhi, Escherichia coli, Pseudomonas aerogenosa, Shigella flexneri, Enterobacter aerogenes, Vibrio cholera, Klebsiella pneumoniae*). [46]
- Antimicrobial potential of dried leaves (HL) and processed powder (HP) of henna confirmed that *Bacillus subtilis* (ATCC6633) was the most sensitive bacteria to both HL and HP extracts with MBC ≈ 165.8 ± 3.7 µg/ml (HP) and 454.3 ± 42 µg/ml (HL) [47]
- Henna has shown antimicrobial activity against four Gram negative (*E coli, Salmonella typhi, Klebsiella spp., Shigella sonnei*) and three Gram positive (*Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*) using disc diffusion method [48]
- Aqueous extract, fractions of ethanol extract and fractionation residue of *Lawsonia inermis* L. leaves possess antibacterial activities against *Staphylococcus aureus*, *Proteus vulgaris*, *Escherichia coli*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *Salmonella typhi* and *Shigella dysenteriae* using Agar-disc Diffusion method [49]
- Henna leaves extracts showed significant antimicrobial activity on all the tested microorganisms (*P. aeruginosa, S. aureus, K. pneumonia, Bacillus* spp., *E. coli, Proteus* spp., and *Enterococcus* spp. with the exception of aqueous extract which showed the least effect on most bacterial samples tested [50]
- *Aqueous*, *ethanol*, *methanol*, ethyl acetate and *chloroform extracts* of the leaves of

Lawsonia inermis Linn. Exhibit antibacterial activity against reference bacterial strains (*E. coli, Klebsiella pneumoniae, Pseudomonas aeuroginosa, Proteus mirabilis, S. typhi, Vibrio cholerae, S. aureus, Methicillin Resistant S. aureus*) and clinical isolates (*Staphylococcus aureus* and Amp C β-lactamases producing *Proteus mirabilis*). It was noticed that the plant material inhibits the growth of AmpC β-lactamases producing *Proteus mirabilis*, which showed resistance to commonly used antibiotics for different infections caused by uropathogens [51].

- Leaves of *Lawsonia inermis* L., showed antibiotic activity against a wide variety of microorganisms—pathogenic and nonpathogenic gram-positive and gram-negative bacteria, yeast, and fungi [52]
- *Methanolic* extract of the leaves of *Lawsonia inermis* Linn inhibit the growth of Gram positive; *B. subtilius, S. aureus* and *S. epidermidis* and Gram negative; *E. coli*, *S. flexneri*, *P. aeruginosa* bacteria in a dose dependent manner using disc diffusion method [53]
- Henna samples from different regions of Oman demonstrated antibacterial activity against a wide range of different bacterial strains with the highest antibacterial activity being demonstrated against *P. aeruginosa* organisms [54]
- In case of *S. aureus*, maximum inhibition of 6 mm from *methanol extract* of *Lawsonia inermis* L [55]
- The ethyl acetate extract of *Lawsonia inermis* L. among the 20 plants species used by Yemeni traditional healers to treat infectious diseases was found to be the most active against all the bacteria in the test system [33,56]
- Red henna (*Lawsonia inermis* L.) and black henna (*Indigo tinctoria*) showed variable antimicrobial activity against tested bacteria and *C. albicans*. Water was less effective than *Oil* and *alcoholic extracts*. *Staphylococcus epidermis* was significantly affected with water extract of black henna (20±1.1) and red henna (11±0.5) [57]
- The *ethanolic extract* of *Lawsonia inermis* leaves showed excellent antimicrobial potency and *Aqueous extract* showed a low activity against the majority of strains tested even with moderate concentrations of extracts (1.5 mg/mL) [58]
- Aqueous and chloroform extract obtained from leaves of *L. inermis* were tested against the primary invaders of burn wounds *viz Pseudomonas aeruginosa, Staphylococcus aureus, Fusarium oxysporum Candida albicans,* and *Aspergillus niger* by using *in-vitro* agar incorporation and well diffusion methods. Extract inhibit growth pattern of all microbes except *C. albicans.* So, it is concluded that henna may be effective in the management of wound infections [59].
- A thermo-stable, proteinaceous protease inhibitor (LPI) reported from *Lawsonia inermis* L. possess antibacterial activity against *Pseudomonas aeruginosa* MTCC 7926 and Staphylococcus aureus NCIM 2079; the IC50 values of the purified LPI were 11.4 µg/mL and 16.6 µg/mL respectively [60]
- Leaf extract of henna (*Lawsonia inermis* L.) for preparation of antibacterial poly (ethylene oxide) (PEO) and poly (vinyl alcohol) (PVA) nanofibers via electrospinning technique were investigated. The study proves that 2.793 wt.% Li in PVA and PEO based solutions showed bactericidal effects against *Staphylococcus aureus* and bacteriostatic action to *Escherichia coli* [61]
- Henna has shown maximum inhibition against *Staphylococcus aureus*, (15 mm) and minimum (13 mm) of *Candida albicans*. The results revealed the presence of important medicinal phyto-chemicals constituents, such as alkaloids, flavonoids, phenols, saponins, α- amino acids, reducing sugar, phlobatannins, tannins and coumarins were present in the plant [62]
- *Hydro-alcoholic* extracts of *Lawsonia inermis* L. has antimicrobial activity against *A. actinomycetemcomitans* [63].

2.4 Antiviral Activity

Unani Physicians have used Henna in the treatment of Erysipelas, Measles and Small Pox. Following studies support their vision for the use of the plant in viral infections despite limited knowledge about the pathology.

- Methanolic extract of *Lawsonia inermis* L. inhibit Sindbis virus at a minimum concentration of 1.5mg/ml. [64]
- At a concentration of 125 micrograms/ml, 13% (6/46) of hot-water extracts, namely

Eugenia caryophyllus, *Phyllanthus urinaria*, *Terminalia belerica*, *Nelumbo nucifera*, *Psidium guajava* and *Lawsonia inermis* had a relative inhibitory ratio (IR) over 50% for their retroviral reverse transcriptase inhibitory activity. They showed ratios of 100%, 91%, 75%, 74%, 61% and 60%, respectively [65].

2.5 Hepato-protective Activity

Unani Physicians have used Henna in the treatment of Liver Diseases. Following studies support the usefulness of the plant as Hepato-protective.

- *Ethanolic extract* of *Lawsonia inermis* dried leaves and its petroleum ether, ethyl acetate, butanol and butanone fractions significantly reduced the total bilirubin content and aspartate aminotransferase or Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic pyruvic Transaminase (SGPT) and Serum Alkaline (SAL) activities, and reduced liver weight compared to Liver Care (LIV-52) used as control [66]
- Butanolic fraction (But-LI) of *Lawsonia inermis* L. leaves was assessed for antioxidant and hepato-protective activity. It was observed that phyto-constituents of *Lawsonia inermis* L. have potential to effectively protect rats from the 2-AAF induced hepatic damage *in-vivo* possibly by inhibition of reactive oxygen species and lipid peroxidation.[67]
- Alcoholic extract of *L. inermis* bark showed hepato-protective activity against the CCl_4 induced hepato-toxicity. It inhibited the elevation in serum marker enzymes (GOT and GPT), serum bilirubin, liver lipid peroxidation and reduction in total serum protein, glycogen, liver glutathione, glutathione peroxidase, glutathione-s-transferase, superoxide dismutase and catalase activity. [68, 69]
- Aqueous extract of *L. inermis* was effective in protecting the liver against the injury induced by Paracetamol in rats. The aqueous extract has significantly reduced the serum enzymes, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alkaline aminotransferase (ALT), Acid Phosphatase (ACP), Protein and Bilirubin. [70, 71]
- The *ethanolic extracts* of the leaves of the *L. inermis* in the dosage of 200 mg/kg p.o. significantly reduced the elevated levels of Serum bilirubin, SGOT, SGPT and SALP when compared to the CCl_4-treated group alone. [72, 73].
- Lawsone isolated from the *L inermis* leaves significantly lowered the serum transaminases levels in the RIF-INH administered animals. The of albumin - globulin ratio was improved and bilirubin level was lowered.[74]
- The two doses of the leaves of *methanolic extract* of *Lawsonia inermis* L. has shown dose-dependent hepato-protective effect. It was evident by the significant reduction in serum levels of bilirubin, AST, ALT, and ALP and improvement in histopathological liver sections compared to CCl_4-only treated animals [75]
- The effects of ethanol extract of the leaves of *Lawsonia inermis* L. on CCl_4-induced liver injuries were investigated. Administration of *extract* not only protected the integrity of plasma membrane of hepatocytes, but also increased the regenerative and reparative capacity of the liver, shown by the significant reductions in the total bilirubin, aspartate aminotransferase, serum alanine aminotransferase, and alkaline phosphatase. It was able to protect and reverse carbon tetrachloride-induced liver injury in Wistar rats [76].
- Treatment with lawsone, a derivative of *Lawsonia inermis* L. to the RIF-INH administered animals significantly lowered the serum transaminases levels. The albumin - globulin ratio was improved and the level of bilirubin was lowered [77]

2.6 Abortifacient Activity

Unani Physicians have described Henna as abortifacient. Following studies support the activity of the plant-

- *Lawsonia inermis* L. root exhibit abortifacient activity [78] The successive ethanolic extract of henna roots in the dosage of 400mg/kg body weight showed promising strong abortifacient and anti-ovulatory activity [79]
- *Lawsonia inermis* L. extract was studied on abortion in pregnant BALB/c mice and it was observed that abortions were more often in the experimental groups. The mean of the serum oestrogen level was significantly higher in the case control

groups and the mean of progesterone level was also significantly lower in the experimental groups [80]

- Methanol extract of *Lawsonia inermis* L. roots was most effective in inducing abortion in mice, rats and guinea pig. The effect apparently was dosage dependent. The results of the whole animal experiments support the *methanol extract* effectiveness as an abortifacient due to its maternal and foetal toxic effects [81]
- It was confirmed that the use of *Lawsonia inermis* L. to induce first trimester abortions, prevent and treat postpartum haemorrhage in traditional medicine and suggests that uterotonic activity involving the beta-adrenergic pathway may be the mechanism [82]
- *Methanol extract* of *Lawsonia inermis* L. was found to be most effective in inducing abortion in dosage dependent manners in experimental animals. The results showed that the *methanolic extract* of *Lawsonia inermis* L. has an abortifacient activity [83]

2.7 Diuretic Activity

- Unani Physicians have mentioned that Henna has diuretic activity. *Aqueous* and *ethanolic extracts* of *Lawsonia inermis* L. leaves in the dose of 250mg/kg and 500mg/kg showed that both the extracts of leaves have significant diuresis, however, *ethanolic extract* showed more activity than *aqueous extract*. [84]

2.8 Antiarthritic Activity

- Unani Physicians have mentioned that Henna has anti-inflammatory as well as analgesic activity and it is used in the treatment of Arthritis. *Ethanolic extract* of *Lawsonia Inermis* Linn (EELI) was studied for anti-arthritic activity against Freund 's Complete Adjuvant (FCA) induced arthritis in rats. It was observed that EELI significantly reduced the paw diameter and gain in body weight. The haematological and biochemical parameters altered in the arthritic control rats were significantly come to normal by the extract treatment at the dose level of 200mg/kg and 400mg/kg. The radiological and histopathological studies have shown the significant anti-arthritic activity of EELI when compared to the arthritic control group. [85,86]
- *Aqueous* and *ethanolic* leaves extract of *Lawsonia Inermis* in the dosage of 200 and 400 mg/kg p.o., respectively showed anti-arthritic activity, as reflected by a reduction in paw oedema, paw diameter and body weight loss in arthritis mice models. In this study, diclofenac sodium in the dose of 10 mg/kg was used as the positive control. [87]

2.9 Wound Healing Properties

Unani Physicians have mentioned that the plant has anti-inflammatory as well as analgesic activities. It is used in the treatment of Boils, Bruises, Burn and Scald.

- *Lawsonia inermis* L.-extracted oil for wound healing in a rat model showed the healing in the test group but not in the untreated group. It showed a full re-epithelialization with re-appearance of skin appendages and well-organized collagen fibres without any inflammatory cells.[88]
- Extract of Henna leaves has remarkable effects wound healing. Histological examination revealed that low dose and high dose produce better wound healing in comparison to control positive and control negative.[89]
- The topical use of the powder of *Lawsonia inermis* L. with honey simultaneously accelerates healing process of the burn wound in rabbit's model. It has also shown a remarkable hair growth promoting activity.[90]
- *Ethanolic extract* of henna leaves and lawsone have shown significant wound healing activity on rat excision and incision wound models. It was observed that the local application of *ethanolic extract* of henna leaves and lawsone were more effective than the same given through oral administration.[91]
- Ethanol Extract of *Lawsonia inermis* L. in the dosage of 200 mg/kg showed high rate of wound contraction, a decrease in the period of epithelialization, high skin breaking strength, a significant increase in the granulation tissue weight and hydroxyproline content. [92]
- *Chloroform* and *aqueous extracts* of leaves of the *Lawsonia inermis* plant inhibited the growth of micro-organisms involved in causing burn wound infections.[93]
- *Lawsonia inermis* L. was experimentally used in the management of wound healing. Histological studies of the tissue showed

the increased well-organized bands of collagen, few inflammatory cells and more fibroblasts, however, control group showed inflammatory cells, fibroblasts and scanty collagen fibres. [94].

- The effect of Henna (*Lawsonia inermis* L.) was evaluated for the wound healing property of local Arabian Horses. It has shown the superiority of the henna extract solution at 20% concentration according to the clinical signs of the wound, i.e. swelling, bleeding, dryness, abscess formation, epithelization and scar tissue formation. The henna extract solution in different concentrations has shown gradually absence of wound edges swelling, no bleeding, dryness, intensive growth of epithelial tissue, no abscess formation and scar tissue since the first week of the treatment. The histopathological examination of the wound has shown strong healing with thick keratinized tissue, hyperproliferation of the epidermal layer, presence of profuse fibrosis and clear growth of new blood vessels when compared with control group [95]
- *Lawsonia inermis* L.-extracted oil was assessed for wound healing properties in a rat model. It was observed that a high content of bioactive components induced an efficient wound healing effect in *in-vivo* study. Healing was observed in the oil-treated group but it was present in the untreated group, Full re-epithelialization and re-appearance of skin appendages was well-organized collagen fibres without any inflammatory cells also observed [96]
- An ointment made from *hydro-ethanolic extract* of *Lawsonia inermis* leaves was evaluated on wound healing by gene expression of glucose transporter-1 (Glut-1) and insulin-like growth factor I (Igf-1) in Wister rats. Topical use of *Lawsonia inermis* shortened inflammatory phase, accelerated cellular proliferation, and enhanced wound contraction ratio in dose dependent manner. It also improved re-vascularization, collagen deposition, and re-epithelialization rate and promoted intracytoplasmic carbohydrate storage [97]
- The extracted oil from *Lawsonia inermis* L. showed antimicrobial effect on Entero-bacter cloacae, anti-yeast effect against Candida parapsilosis and Candida sake, and antifungal activity against three opportunistic cutaneous molds including Penicillium, Aspergillus, and Fusarium. [97]

2.10 Memory Enhancing Properties

Unani Physicians have mentioned that the plant has brain tonic activity. It is used in the treatment of boils, bruises, burn and scald.

- *Lawsonia inermis* L. ethanol (Li. Et) and chloroform (Li. Chf) extract in the dosage of 25 mg/kg (p.o.) have improved learning and memory significantly. Li. Et and Li. Chf at 25 mg/kg (p.o.) showed considerable increase in catalase (CAT), glutathione (GSH), and superoxide dismutase (SOD) levels as compared to positive and negative control groups. [98]
- Memory enhancing activity of the leaves extract of *Lawsonia inermis* Linn. was evaluated against scopolamine induced amnesia by elevated plus maze and Y-maze test. The result showed the presence of phenolics, tannins, flavonoids and a potent *in-vitro* free radical scavenging activity. These findings confirm the memory enhancing effect of *Lawsonia inermis* L. leaves, which may be due to enhancement of cholinergic neuro-transmission through inhibition of AChE activity and by stabilizing the antioxidant system. [99]
- Extract of *Lawsonia inermis* L. (Henna) leaves has significant prophylactic effect on neuro-degenerative disease animal model of Alzheimer's Disease, which indicates the abilities to improve memory and reduce neuronal cell death by inducing Nissl bodies / Ribosome proliferation and increasing anti-oxidant enzyme status.[100]
- The crude extract (CE), 50% MeOH, Petroleum Ether (PE) and Ethyl acetate (EA) fractions of the leaves of the *Lawsonia inermis* L. were evaluated for their antioxidant activity and their ability to counteract amyloid-β42 (Aβ42) aggregation. A new compound namely 1,2,4-trihydroxynaphthalene-2-O-β-D-glucopyranoside (THNG) was characterised with powerful antioxidant and anti-Aβ42 aggregation properties. This compound has a strong co-relation with the antioxidant and anti-Aβ42 aggregation activities of plant leaves.[101]
- Ethanol (Li.Et) and chloroform (Li.Chf) extracts of *Lawsonia inermis* L. were

assessed as memory-enhancing agents by using two methods including "without inducing amnesia" and "induction of amnesia" by administration of diazepam (1 mg/kg, intraperitoneally. Piracetam at 400 mg/kg (i.p.) was used as positive control in experimental animals. It was observed by Cognitive behavioural models and Biochemical markers of oxidative stress such as glutathione (GSH), catalase (CAT), superoxide dismutase (SOD) levels that *Lawsonia inermis* L. may be used as memory-enhancer and antioxidant compounds.[102]

- Aqueous extract of three ecotypes of henna (Shahdad, Roodbar and Bam) was investigated against two types of Gram positive and Gram negative bacteria, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Bacillus cereus*, *Corynebacterium pseudotuberculosis*, *Klebsiella pneumonia*. The plant extract prevents the growth of bacteria in a dose dependent manner. The species of *K. pneumonia* and *B. cereus* has shown higher resistant to henna *aqueous extract* when compared with other bacteria species. *Aqueous extract* of the leaves of Shahdad ecotype had the highest antioxidant activity in comparision to other ecotypes.[103]

2.11 Anti-ulcer Activity

Unani Physicians have mentioned that the plant has anti-inflammatory as well as analgesic desiccation of ulcer activities and used in the treatment of urinary tract ulcers.

- Aqueous, ethanol and chloroform extracts of Henna leaves showed a strong anti-ulcer activity in pylorus ligation- and aspirin-induced rats, when compared to ranitidine, the positive control. In gastric acid secretions, total acidity and ulcer index were significantly reduced.[104]
- Significant of the combination of *Ethanolic extract* of *Lawsonia inermis* L. and *Murraya koenigii* seeds was evaluated for anti-ulcer activity and it was observed that development of gastric ulcers was prevented in ethanol induced rats. It has also shown significant protection in comparison to standard [105]
- The test formulation comprising *Azadirachta indica* & *Lawsonia inermis* L. has reduced the ulcer area significantly in Naproxen induced model, which is very close to standard [106]
- Extract of *Lawsonia inermis* L. has shown a significant gastro-protective effect in high dosage. The volume of gastric secretion was decreased significantly by *L. inermis* (42.8%) similarly to omeprazole's anti-secretory effect (50% of secretion reduction) [107]
- *Aqueous, ethanolic* and *chloroform extracts* of *Lawsonia inermis* L. were found to reduce ethanol-induced ulcers by up to 81, 94 and 88% and cold-restraint stress-induced ulcers by up to 56%, 30% and 56%, respectively [108]
- *Ethanolic extract* of *Lawsonia inermis* leaves has shown anti-ulcer activity in indomethacin-induced gastric ulcers in pylorus ligation rat models by reducing the ulcer index the tested dosage of 100, 200 and 400 mg/kg p.o [109].
- A Randomized Clinical Trial (RCT) was carried out to determine the cooling and protecting effects of henna on the prevention of decubitus ulcers in critical care units. At the end of the study, one patient in the intervention group (2.7% male) and six patients in the control group (14.29% male, 2.85% female) had developed decubitus ulcers, this was a significant difference. Application of henna as a preventive measure may be recommended for the patients at the risk of developing decubitus ulcers [110]

2.12 Vitiligo

- Unani Physicians have mentioned that the plant is used in the treatment of vitiligo. The *methanolic* and *aqueous extracts* of all four plants including *Cassia fistula*, *Eclipta alba*, *Plumbago zeylanica*, *Lawsonia inermis L.*, and *Indigofera tinctoria* have shown the presence of phenols, flavonoids, tannins, alkaloids, terpenoids and steroids in the extracts. The aqueous extract of all these plants can be used in the treatment of Vitiligo as easily accessible source of natural antioxidants.[111]

2.13 Stomatitis

- Unani Physicians have mentioned that the plant is used in the treatment of stomatitis. The effect the extract of *Lawsonia inermis* L. on *S. mutans* and *L. acidophilus* as

plaque inducer and pathogenic bacterium was evaluated. The study showed a high activity of the extract against *L. acidophilus* and *S. mutans.* Results has also shown decrease in *S. mutans* growth with the increase of *Lawsonia inermis* L. extract concentration. Result have also shown that even the highest *Lawsonia inermis* L. extract concentration does not completely inhibit *S. mutans* growth but bacterial growth with 50% extract was very low. [112]

2.14 Anti-asthmatic Activity

Unani Physicians have mentioned that the plant is used in the treatment of cough. *Methanolic extract* of aerial parts of *Lawsonia inermis* L. has shown significant antihistaminic (H1 receptor antagonist) activity. It has also shown antioxidant, anti-inflammatory, Broncho-dilator properties and therefore can be used in the treatment of bronchial asthma [113]

3. ADDITIONAL RECENT STUDIES

3.1 Anti-cancerous Activity

- The effect of a naphthoquinone (5-hydroxy-2-methyl-1, 4-naphthoquinone) namely plumbagin present in *Lawsonia inermis* was evaluated on B16F10 murine melanoma cells. It was observed that plumbagin has an anti-invasion and anti-metastasis effect on melanoma cancer cells by acting on MAPK pathway and its related genes. [114]
- The study was conducted to design nano - niosomes containing Lawsone (Law) using non-ionic surfactants and cholesterol. *In vitro* study has shown that using of niosome to encapsulating Law significantly increases the antitumor activity of formulation in MCF-7 cell line compared to Law solution (free Law). [115]

3.2 Anti-Dermatitis Activity

- A randomized, double-blind, placebo-controlled clinical trial was conducted to assess the efficacy of a topical preparation of henna in management of contact dermatitis in patients using lower limb prosthetics. It was observed that there was a significant improvement in the symptoms of contact dermatitis including skin edema, sweating, itching, skin thinning and pain (p-value<0.05) in the test drug group in comparison to the placebo group. It was also observed that skin redness was significantly increased in the henna group [116].

3.3 Anti-Psoriatic Activity

- A study was carried out to develop and assess the potential of Nanostructured Lipid Carriers (NLCs) enriched with the powdered extracts of the leaves of *Lawsonia inermis* L. (LE) in the management of psoriasis. The LE loaded NLCs has shown to possess anti-lipid peroxidation and nitric oxide scavenging activities, cytoto-xicity on HaCat cell lines, DNA fragmentation on HaCat cell lines, which are the biomarkers in the pathogenesis of psoriasis. The results confirm the anti-psoriatic potential of *Lawsonia inermis* L. (LE) [117].

3.4 Dyeing Properties

- The study was done to evaluate the effect of the herbal hair dye derived from *Lawsonia inermis* L. (henna). The result has shown a positive impact on the cuticula pattern and on the diameters of the examined samples, after henna application [118].
- Henna extract-loaded micro-capsules were prepared with an average particle size of 4.1 μm by excellent encapsulation efficiency (98.4%) and a negative zeta potential (−34.4 mV). The *in vitro* toxicological studies showed that the micro-encapsulation process significantly reduced the contact toxicity of henna extract towards human epidermal keratinocytes as well as to the skin and eyes [119].
- A new organic-inorganic hybrid pigment based on a natural chromophore. Lawsone was selected as the active organic compound and incorporated into aluminum-magnesium hydroxyl-carbonate (LH). Dynamic mechanical analysis (DMA) and the results of tensile break tests showed that the EN+LH / lawsone composite had significantly better resistance to solar irradiation in comparison to EN and EN with an unmodified carrier [120].

3.5 Phytochemical Constituents

The preliminary phytochemical analysis reveals that the *aqueous* and *petroleum ether extracts* from leaf of *Lawsonia inermis* L. consists of alkaloids, anthraquinones, flavonoids, phenols, reducing sugars, saponins, steroids, tannins, and terpenoids.

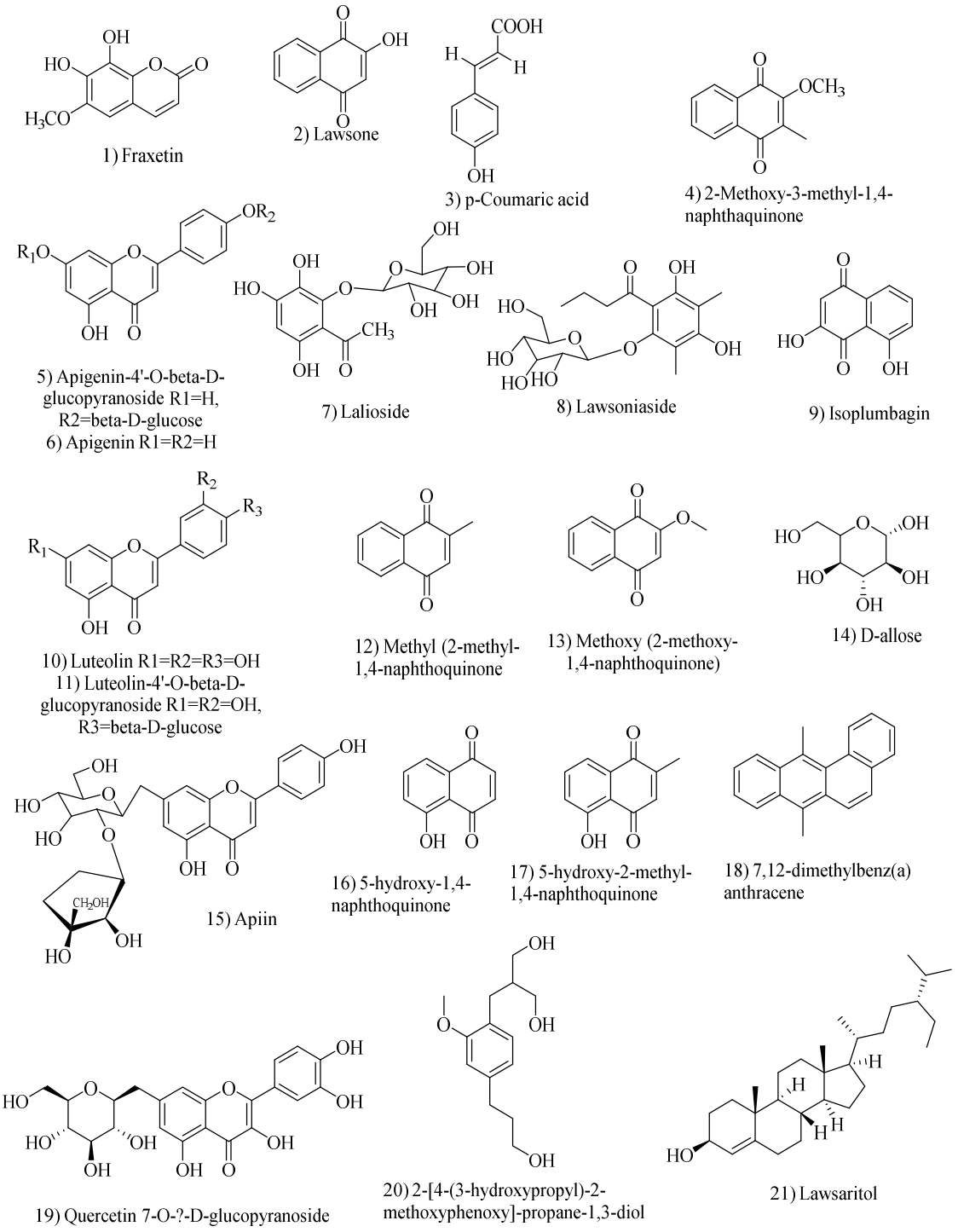

Fig. 1. Chemical structure of phyto-constituents of *Lawsonia inermis* L.

Table 3. Reported pharmacologically active phyto-constituents of *Lawsonia inermis* L.

Pharmacological activity	Reported constituents	References
Antifungal Activity	2-hydroxy-1,4-naphthoquinone (Lawsone)	[121], [122]
Anti-Inflammatory, Analgesic and Antipyretic Activity	Lawsone, isoplumbagin, lawsaritol, fraxetin, Lawsochylin A, luteolin, apigenin, 4-hydroxy-α-tetralone, 2-butoxysuccinicacidandlawsonaphthoateA	[123], [121], [124]
Antibacterial activity	2-hydroxy-1,4-naphthoquinone, 5-hydroxy-1,4-naphthoquinone, methoxy (2-methoxy-1,4-naphthoquinone), methyl (2-methyl-1,4-naphthoquinone, and 5-hydroxy-2-methyl-1,4-naphthoquinone	[125]
Antiviral Activity	Luteolin	[126]
Abortifacient Activity	D-allose	[127]
Antiarthritic Activity	Lawsone	[123]
Memory enhancer	2-[4-(3-hydroxypropyl)-2-methoxyphenoxy]-propane-1,3-diol (1) and quercetin 7-O-β-D-glucopyranoside	[128]
Wound Healing Properties	NA	
Anti-ulcer Activity	NA	
Stomatitis	Lawsone	[125]
Anti-asthmatic activity	NA	
Anti-cancerous activity	7,12-dimethylbenz(a) anthracene	[129]
Anti-Dermatitis activity or Anti-Psoriatic activity	NA	
Dyeing properties	2-hydroxy-1,4-naphthoquinone	[130]
Antileishmanial activity	2,4,6-trihydroxyacetophenone-2-O-β-D-glucopyranoside, lalioside, luteolin-4′-O-β-D-glucopyranoside, apigenin-4′-O-β-D-glucopyranoside, luteolin, and apigenin.	[131]
Immunomodulatory	p-coumaric acid, 2-methoxy-3-methyl-1,4-naphthoquinone and apiin	[132]

4. CONCLUSION

It is concluded that Unani Physicians have mentioned different pharmacological actions of Henna (*Lawsonia inermis* L.) and used it in the treatment of different ailments, which were studied for pharmacological actions and phytochemical constituents on modern scientific parameters. Detailed analysis of the outcomes of different studies gives a firm cause to recommend the use of *Lawsonia inermis* L. It also indicates that actions and uses of henna mentioned by ancient Unani Scholars gained with vast experience have some basis. Thousands of drugs, single as well as compound/regimen have been mentioned by Unani physicians for different disorders, which may be investigated scientifically especially in the field where limited medicines are available at present. This may benefit the masses in addressing the unanswered health issues.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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