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A COMPLETE REVIEW ON HEENA LAWSONIA INERMIS PLANT AND LAWSONE AS ITS COLOURING COMPONENT

Jain A., Fulmali S.* and Rajput Tanya

Shri D. D. Vispute College of Pharmacy & Research Center, New Panvel.

***Corresponding Author: Dr. Fulmali S.**

Shri D. D. Vispute College of Pharmacy & Research Center, New Panvel.

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ABSTRACT

Henna is one such plant commonly known as Persian Henna or *Lawsonia inermis*, a bushy, flowering tree, commonly found in India. Leaves of henna contain Lawsone the chief active ingredient responsible for its dye properties. The dried leaves paste is used as cosmetic for decoration of hand, feet and body on different festivals and religious occasions in India, as hair dye and hair conditioner to improve their lustre and also extensively used as a dye in silk and wool industries. Normally the concentration of Lawsone founded in leaves between 0.5 to 1.5%. But physical conditions influence on the dye properties and percent of Lawsone. Henna leaves also contain mannitol, tannic acid, mucilage, gallic acid, and napthaquinone. The flower of Henna has a strong aroma with high commercial value. Several researchers have reported the different biological actions like antioxidant activity, Wound Healing activity, Antibacterial activity, Immunomodulatory activity etc. Henna contains lawsone /hennotanic acid, carbohydrates, phenolic compounds, flavonoids, saponins, proteins, glycosides, alkaloids, terpenoids, quinones, coumarins, xanthones and 6% fats, 2-3% resins, 7-8% tannins. Hence this plant is very useful and need to be commercialisation for herbal colourant.

KEYWORDS: Lawsone, Immunomodulatory activity, Napthaquinone.

1. INTRODUCTION

Lawsonia inermis of family Lythracae, Commonly known as Henna is a perennial shrub. It was believed to have originated in North Africa (Egypt Arid Area perhaps Ethiopia) and has naturalized and cultivated in the tropics of America, Egypt, India and part of middle east. It is also known as Elhenna, Egyptian priest, and Mignonette Tree. Henna is a large shrub reaching a height of up to 6 meters. It has spreading lateral branches with opposite leaves.[1]

Leaves of henna contain Lawsone the chief active ingredient responsible for its dye properties. The dried leaves paste is used as cosmetic for decoration of hand, feet and body on different festivals and religious occasions in India, as hair dye and hair conditioner to improve their lustre and also extensively used as a dye in silk and wool industries. Normally the concentration of Lawsone founded in leaves between 0.5 to 1.5%. But physical conditions influence on the dye properties and percent of Lawsone. Henna leaves also contain mannitol, tannic acid, mucilage, gallic acid, and napthaquinone. The flower of Henna has a strong aroma with high commercial value.[2]

Henna contains natural ingredients which are vital for nourishment of hair. It has a bond with the hair structure as it serves to penetrate, cleanse and thicken the hair thus improving its quality. It also has great dandruff fighting ability. Henna is mainly used as a colouring agent. Nature has been a rich source of therapeutic agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources based on the uses of these plants in traditional medicine.

Henna is one such plant commonly known as Persian Henna or *Lawsonia inermis*, a bushy, flowering tree, commonly found in India. It is the most common forms for medicinal benefits, and the high concentration of chemicals and nutrients in the plant gives it anti-inflammatory, hypotensive, antibacterial, astringent, and antiviral agent, among many others. It has antioxidant capacity of henna has not been widely studied, the oil has been proven to be an astringent, which has led some people to use henna juice and oil on the skin to reduce the scars and signs of aging and wrinkles. It shows antiviral and antibacterial activity that can protect the body's largest organ is the skin. Henna oil is used for rheumatic and arthritic pains. Ground leaves are applied to ease rheumatism. It juice of the medicinal plant can be applied to the skin for headaches, and the henna oil is applied to hair to prevent it from greying.[3]

1.1 History of henna plant

Henna has been used cosmetically and medicinally for over 9,000 years. There exits a knowledge, information and benefits of herbal Drugs in our ancient literature of Ayurvedic (Traditional Indian Medicine), Siddha, Unani and Chinese medicine. According to the World Health Organization, 2003 about 80% of the population of developing countries being unable to Afford pharmaceutical drugs rely on traditional medicines, Mainly plant based, to sustain their primary health care needs.[4] The art of Henna, which is the art of painting some part of the body by using henna leaves plant, has been recognized For many centuries to the communities in Asia and Africa.[5]

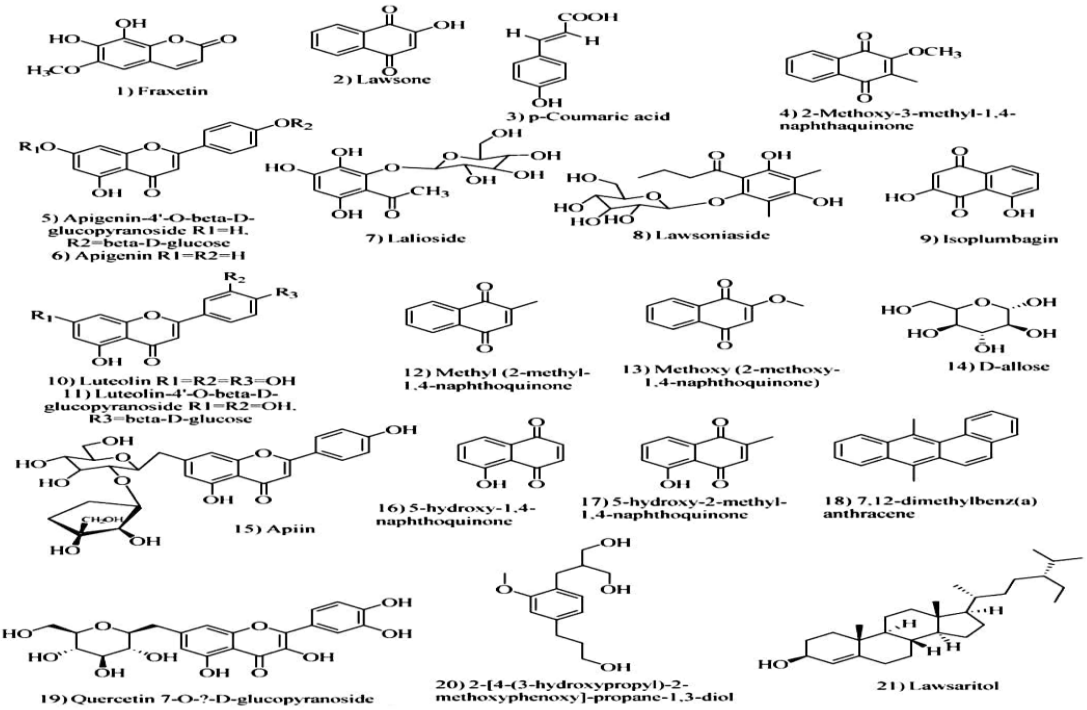
The Unani Medicine is a comprehensive medical system, which deals with the various states of health and disease. It provides promotive, preventive, curative and rehabilitative healthcare. The fundamentals, diagnosis and treatment modalities of the system are based on scientific principles and holistic concepts of health and healing.[3] Unani scholars retain its traditional strength successfully and also benefitting from scientific development over the years. The system fully appropriate the validation of existing knowledge on modern scientific parameters as well as new action of existing drugs were also discovered.[6]

Henna was used for cosmetic purposes in the Roman Empire, Convivencia-period Iberia and Ancient Egypt, as well as other parts of North Africa, the Horn of Africa, the Arabian Peninsula, the Near East and South Asia. It can be found widespread in other hot climates like Pakistan, India and Australia. The plant grows best in heat up to 120F degrees and contains more dye at these temperatures. Inversely, it wilts in temperatures below 50F degrees.[5] Henna is a tropical plant native to Old World (Africa, Asia and India). Vedic and Buddhist India used henna for medicinal purposes, fingernail dye, and hair dye. Ancient Indian texts, pre-Islamic Arabic and Persian texts, and artifacts have body markings consistent with henna. Evidence in the Ajanta caves at 400 CE indicates that men and women used henna to paint feet and hands.[7]

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. 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.[8] 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.[7]

1.2 Phytoconstituents in henna plant

Different chemical constituent in Henna plant that has pharmacological importance:



1.3 Pharmacological activities of henna plant

Several researchers have reported the different biological actions of L. inermis in various in-vitro and in-vivo test models. Henna leaves, flower, seeds, stem bark, roots have been found to exhibit antioxidant, antidiabetic, hepatoprotective, hypoglycemic, antimicrobial, anticancer and wound healing properties. These are described in greater details in the following sections.

1. Antioxidant activity

Lawsone (2-hydroxy-1, 4 napthoquinone) is the mainingredient of L. inermis. On oxidation of 100 µM phenanthridine by guinea pigs aldehyde oxidase, superoxide anion and hydrogen peroxide formation was found to be 6-10 % and 85-90 % respectively. The mechanism of action was believed to be flavinsemiquinone (FADH).[9][10] The hepatic glutathione S-transferase and DT-diaphorase specific activities were elevated above basal level by L. inermis extract treatment.[8]

2. Wound healing activity

Extract of L. inermis 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. These fundings suggest the use of L. inermis in the management of wound healing.[9]

3. Antibacterial activity

Henna showed strong against Bordetella bronchi septica. These findings indicated that L. inermis can be used in the treatment of bacterial infections.[13] Henna dry leaves demonstrated the best in-vitro antimicrobial activity and in particular against Shigella sonnei . Ethanol extracts of 20 plants species used by Yemeni traditional healers to treat infectious diseases were screened for their antibacterial activity.[10]

4. Antiparasitic activity

During an ethnopharmacological survey of antiparasitic medicinal plants used in Ivory Coast, 17 plants were identified and collected. Polar, non-polar and alkaloidal extracts of various parts of these species were evaluated in-vitro in an antiparasitic drug screening.[11]

5. Antifertility activity

Ethanol extract prepared from the powdered seeds of L. inermis failed to show significant antifertility activity. However in subsequent studies it was observed that the powdered leaves of henna, when administered as suspension or incorporated into the diet inhibited the fertility of rats. The fertility induced appeared was found to be permanent.[12]

6. Immunomodulatory activity

Methanol extract of henna leaves at 1 mg/ml concentration had displayed immunostimulant action as indicated by promotion of T-lymphocyte proliferative responses. Seven compounds were isolated adopting the lymphocyte transformation assay (LTA)-guided fractionation of the total methanolic extract of henna leaves.[13]

7. Antidiabetic activity

Ethanol (70 %) extract of L. inermis showed significant hypoglycemic and hypolipidemic activities in alloxan induced diabetic mice after oral administration. The feeding of 0.8 g/kg of L. inermis extract decreased the concentration of glucose, cholesterol and triglycerides to normal.[14]

8. Anticancer activity

The anticarcinogenic activity of chloroform extract L. inermis leaves was carried using microculture tetrazolium salt assay on the human breast (MCF-7), colon (Caco-2), liver (HepG2) carcinoma cell lines and normal human liver cell lines.[15]

1.4 Monograph of henna plant

Synonyms- *lawsoniaalba, lawsoniainermis*
Common names:
English: Henna
Sanskrit: nil. Madayantika
Hindi: Mehandi
Marathi: Mendi.[16]
Taxonomical classification
Kingdom: Plantae.
Family: Lythraceae.
Genus: Lawsonia.
Species: *Lawsoniainermis.*[17]
Dose: 5-10 ml.
Description:[16][18]

A) Macroscopic

Leaves simple, 2 to 3 cm in length, 1 to 1.5 cm in width, greenish brown to dull green; entire, lanceolate; apex micronate, base tapering, petiole short and glabrous.
Odour: aromatic when crushed.
Taste: sweet, slightly astringent.

B) Microscopic

Petiole – shows concavo – convex outline; epidermis consisting of single layered cell covered by thick, striated cuticle, below epidermis 2-4 layer collenchyma and 3-4 parenchyma having intracellular spaces. Phloem consisting of usual elements; xylem mostly composed of tracheids and vessels.

Midrib – shows upper and lower epidermis covered externally by thick and striated cuticle; epidermis followed by 2 to 4 layers of collenchymatous cells, circular in shape with angular thickening; beneath which are 3 or 4 layers of parenchymatous cells, isodiametric with intercellular spaces; stele crescent-shaped, consisting of usual elements traversed by medullary rays; phloem fibres seen in the phloem region; a few parenchymatous cells contain rosette and prismatic crystals of calcium oxalate.

Lamina – shows upper and lower epidermis composed of tangentially elongated cells covered externally by a thick striated cuticle; some large epidermal cells form mucilage sacs projecting into adjacent palisade zone; anomocytic stomata distributed on both surfaces; mesophyll composed of 1 to 3 layers of palisade tissue and 2 to 4 layers of spongy parenchyma; palisade cells filled with chloroplasts, spongy parenchyma oval to circular in shape, oil globules present in palisade and spongy parenchyma; rosette and prismatic crystals of calcium oxalate also present in spongy parenchyma; mesophylle traversed by vascular strands composed of xylem surrounded by phloem with a patch of sclerenchymatous fibres on abaxial side; average stomatal index: upper surface -10 to 15 and lower surface- 15 to 18.Pallisaderatio: 5 to 8 on both surfaces. Vein islet number 30 to 45.

Powder: Greenish dark brown, anomocytic stomata, Rosette and Prismatic crystals of calcium oxalate, a few oil globules

Chemical constituents[19][20]

Henna contains lawsone /hennotanic acid, carbohydrates, phenolic compounds, flavonoids, saponins, proteins, glycosides, alkaloids, terpenoids, quinones, coumarins, xanthones and 6% fats, 2-3% resins, 7-8% tannins.

Pharmacological effects

Antibacterial, antifungal, antiparasitic, antioxidant, hepatoprotective, analgesic, anti inflammatory, antipyretic, wound and burn healing, antiulcer, antidiabetic, anticancer and many other pharmacological effects.

Traditional use[17]

Leaves of lawsonia inermis provide an important cosmetic dye. Henna leaves were extensively used for centuries in the middl east, the far east and northern Africa as dye for nails, hands, hair and textile. Henna was also used in treating skin problems, headache, jaundice, amebiasis and enlargement of the spleen

1.5 Historical development of lawsone[21][22]

Lawsone was discovered by DuPont and is under development by Agro-Kanesho Co. Ltd. And Tomen Agro. It is described by Kinoshita et al. (1999) and Wakasa and Watanabe (1999). Several plant dyes which are rich in naphthoquinones such as lawsone from henna, juglone from walnut and lapachol are reported to exhibit antibacterial and antifungal activity. Gupta et al. have studied the antimicrobial properties of eleven natural dyes against Gram-positive and Gram-negative bacteria.

Lawsone is hypothesized to undergo a reaction similar to strecker synthesis in reactions with amino acids. Recent research has been conducted on lawsone's potential applications in the forensic science field. As of now the research is inconclusive, but optimistic. Lawsone non-specifically targets primary amino acids, and displays photoluminescence with forensic light sources.

1.6 Monograph of lawsone

Synonyms: hennotannic acid, 2- hydroxy-1,4-naphthaquinone.
Biological source: lawsone is the active constituent obtained from henna or *lawsonia inermis*. Lawsone, a major colorant extracted from dried or fresh leaves of *lawsonia inermis linn.*
Family: lythraceae.[23]

Properties: lawsone is soluble in dichloromethane, methyl acetone, isopropyl alcohol, chloroform, ethyl acetate, diethyl ether & insoluble in water.
Formula: $C_{10}H_6O_3$
Category: antimicrobial
Ld_{50}: 100 mg
Appearance: yellow powder
Molecular weight: 174.15.
Melting point:190°c.[24]
Structure:

Uses[25]

- Lawsone has antimicrobial property.
- Lawsone is used to dye wool and silk in orange shade.
- The dye has also long been used by the people, especially the ladies of India and the middle eastern countries for tinting finger nails and the palms of their hands a reddish-brown, dyeing the hair and eyebrows and for other forms of personal adornment.
- Several researchers have reported the different biological actions of *l. inermis* in various in-vitro and in-vivo test models.
- Henna leaves, flower, seeds, stem bark, roots have been found to exhibit antioxidant, antidiabetic, hepatoprotective, hypoglycemic, antimicrobial, anticancer and wound healing properties. These are described in greater details in the following sections.
- They have also shown ability to heal wounds, burns and hemorrhoids via proliferation of granulation tissues

Storage
Store protected from moisture.

1.7 Extraction of lawsone[26]

As per reported there are three main extraction methods for extraction of lawsone:

1. Soxhlet Extraction.
2. Maceration in water.
3. Tommasi method.

Soxhlet is a standard extraction method that has been reported in many research article. It is as well the most widely used technique for the extraction of the natural products from solid phase. In soxhlet Ashnagar A. et. al has reported procedure for extraction of lawsone by the method I. e. soxhlet extraction. In macertion process, the whole or coarsely powdered crude drug is simply placed in beaker containing water as solvent and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved. Then collect the extract of henna. This method was reported by author Ashnagar et. al. for the extraction of lawsone from henna. In Tommasi method, the powders or leaves placed in distilled water and then suspension was stirred on magnetic stirrer with heating and maintain the temperature at around 80°C. this procedure for extraction of lawsone from henna was reported by author Shiri A. et. al. This methods gives more yield as compare above methods.

1.8 Synthesis of lawsone[27][28]

Synthesis of 1-phenylazo-2-naphthol was reported by author Catino S.C. et. al. 4 ml of aniline dissolve in 16 ml of conc. HCl and add 11 ml distilled water shake to dissolve and cool to 5°C then add 4g sodium nitrate dissolve in 20 ml of water and add 1 spatula of urea (5°C). Then diazotiation is achived by adding cold solution of sodium nitrile to cold solution of aniline with stirring and temperature maintain 5°C. Solution of 2-napthol was prepared by dissolving 2-napthol (5g) of 10% NaOH in 250ml beaker with constant stirring. Synthesis of 1-amino-2-naphthol hydrochloride from 1-phenylazo-2-naphthol was reported by author Catino S.C. et.al. were crude uncrystallized (1-phenylazo-2-napthol) 8g dissolved in beaker containing 60 ml methylated spirit. Then it is poured into RBF fitted with reflux condenser. Then mixture was boiled until azo compound get dissolved. Solution of Tin(II) chloride (20g) dissolve in 60ml of conc HCl to produce clear solution and reflux for 30min. then Poured solution in beaker placed in ice bath for cooling the crystals appeared. Then filter crystals and recrystallized with hot water that contain 2 drops of Tin(II) chloride sol and equal weight of HCl. 15-30g of crystals of (1-Amino-2-napthol HCl) collected. Synthesis of 1-Amino-2-napthol-4-sulfonic acid is reported by author Fieser L.F. weigh 1.5g of 1-Amino-2-napthol HCI and 6g of sodium bisulfide in 100 ml of sodium hydroxide and then mix the sol and dilute to 40-50 ml until it get dissolved. Then solution is stored in 400ml conc. H2SO4 (and avoid from sunlight). temperature rises from 20-25°C to 35-40°C at once and 50°C in 2 hrs. After standing for 5 hours precipitate form wash, filter the crystals and 10-25 g of 1-amino-2-napthol-4 sulfonic acid crystals obtained. Synthesis of Lawsone from 1-amino-2-napthol-4-sulphonic acid was reported by Catino S. C. et. al. in last step 1L of NaOH at temp 0°C add 80 ml of conc. H2SO4 and then freeze it. And 1g of (1M) 1-amino-2-napthol-4-sulfonic acid is added and then allow to stand for 30 min temp rises at 15-20°C. Heat gradually on steam bath with shaking till sol boils to 15 min. solution become red and begin separate and filter through buchner funnel and wash with cold water until filtrate nearly colourless. then 5-10g of crystals are obtained (Lawsone).

1.9 Purification of lawsone by column chromatography[29][30]

The most significant method for the purification is column chromatography for lawsone and thw solvent reported is chloroform: petroleum ether: acetic acid (4:6:0.5). Methods used for lawsone purification is dry packing, the stationary phase i.e. silica of 60 mesh size is deposited in the column before the solvent. Then filling the column to the intended height with the stationary phase. Then slowly addition of the nonpolar solvent. The most important aspect of packing the column is creating an evenly distributed and packed stationary phase. The

packing is completed the solvent is loaded into the column without disrupting the packing of column & equilibrated for 24hrs. Minimum 5 to 10 drops is used to dissolve the sample into the solvent. The sample is loaded with the help of prepared from the side of column. Then Collecting small fractions (1-3 mL) is important to the success for column separation. The mixture to be separated contains colored compounds. The colored bands will move down the column along with the solvent and as they approach the end of the column, collect the colors in individual containers. Use the color as guide. After all the materials have been removed from the column, the colors of the materials results should indicate which fractions contain the compound isolating i.e. Lawsone.

1.10 Analysis of lawsone

The Reported analytical method of lawsone was used by various author. Nijsiri.R. et.al has described TLC of lawsone for proper characterization as TLC is a highly versatile method used for of sample. It is quick, sensitive, and inexpensive technique used to determine the number of components in a mixture, it's identity, purity of a compound, determine by mobile phase used as butanol, ethyl acetate, and acetic acid in ratio (7.5:2.5:0.1) and Rf value was reported to be 0.71.[31] Author Khorrami.J.S et.al has reported the analysis of lawsone by colorimetry method which shows the intensity of colour in samples. It is light-sensitive tool used to measure the absorption and transmission of light passing through a sample solution and the reported result obeys beer's lambert law resembling linear graph.[32]

Author JunjieXiang. et.al reported the analysis of Lawsone by FTIR for detecting functional groups and characterizing covalent bonding information, the reported result of Junjie Xiang et. al shows the characterisation of lawsone as Medium, sharp (O-H) peak at 3500cm^{-1}, medium (C-H) peak at 2200cm^{-1}, strong (C-O) peak at 1210cm^{-1}, strong (C=C) at 1650cm^{-1}. It is an effective analytical instrument used for study of the interaction between matter and electromagnetic radiations.[33]

Author MengCK. et.al reported the analysis methods by LCMS for separation, identification, and quantification of both unknown and known compounds as well as chemical properties of different molecules. LC-MS technology involves use of an HPLC, wherein the individual components in a mixture are first separated followed by ionization and separation of the ions on the basis of their mass/charge where the lawsone molecular mass peak was given to be 174.[34]

Author Souney P.F et.al reported the analysis of Lawsone by UV spectroscopy which studies that it is an analytical technique that measures the amount of discrete wavelengths of UV or visible light that are absorbed by or transmitted through a sample in comparison to a reference or blank sample. Mainly depend on the principle of absorption and on the absorption spectra mode the lawsone shows the maximum absorption at 452nm.[35]

CONCLUSION

Lawsone is very important component of heena plant. It can be synthesise chemically but the process of synthesis is very long. So to obtain this colouring component plant is best source as it will protect environment as well as good quantity can be obtained from heena plant.

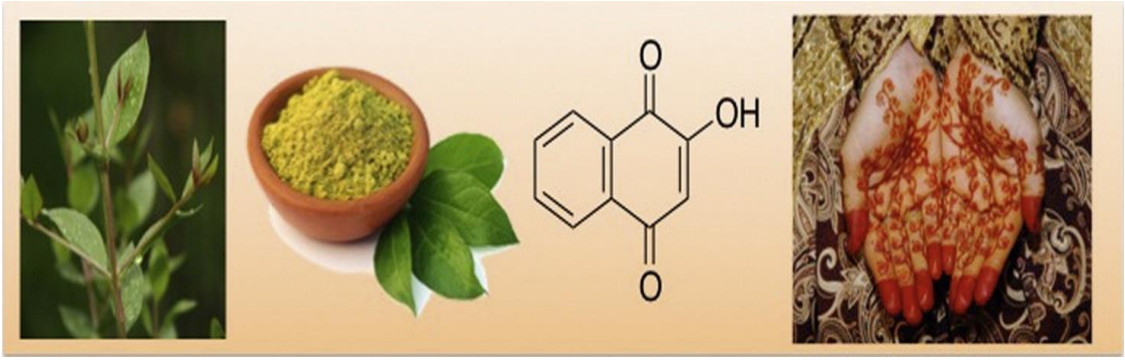


Fig. no. 1:- Natural source, Structure and Use of lawsone.

Fig. No. 2:- Lawsone crude powder.

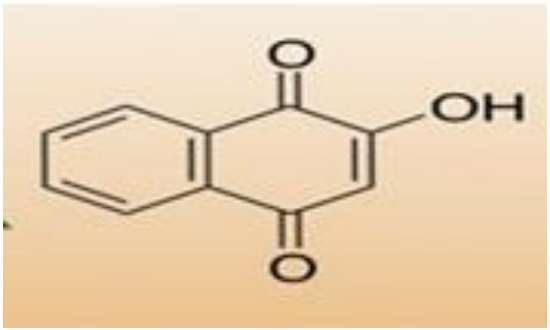


Fig. no. 3:- Structure of lawsone.

Table no. 1:- Physical phytoconstituents screening.

Parameter	Standard Value
Total ash	Not more than 11 percent
Acid insoluble ash	Not more than 3 percent
Alcohol soluble extractives	Not less than 18 percent
Water soluble extractives	Not more than 25 percent

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