Exploring Lawsonia inermis: A review of its role in Herbal medicine and modern pharmacology

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***Abstract*—This is native to subtropical regions worldwide. This shrub typically grows between 2 and 7 meters in height and features smooth bark with young, quadrangular, cylindrical branches. Henna has been used for centuries in various cultures, with its medicinal properties being well-documented in traditional medicine, particularly in India. The plant contains several bioactive compounds, including glycosides, phytosterols, steroids, saponins, tannins, flavonoids, terpenoids, cardiac glycosides, and 2-hydroxy-1,4-naphthaquinone, all of which contribute to its therapeutic effects. Henna seeds are known to be effective in treating dysentery and diarrhea, while the flowers are used to alleviate fever and psychosis. In addition to its medicinal uses, henna has a strong presence in cosmetic applications, particularly in hair and skin care products, where it is often used to treat dandruff and other scalp conditions. In some African cultures, henna is also regarded as an abortifacient.**

***Index Terms*—*Lawsoniainermis*, Henna, Traditional medicine, Constituents, Therapeutic potential, Toxicity.**

I. INTRODUCTION

The plant Lawsoniainermis Linn (henna) is a member of the Lythraceae family and is mostly found in subtropical areas worldwide [1]-[3]. Usually growing between 2 and 7 meters tall, Lawsoniainermis is a shrub or small tree with smooth bark and young, quadrangular, cylindrical branches [4]. In Indian medicine, lawsonia has been used to treat amenorrhea, dysmenorrhea, skin conditions, and leprosy. Henna leaves are used to cure anemia, rheumatism, coughs, bronchitis, wounds, ulcers, and one-sided headaches. The flowers are used to treat acute psychosis, fever, and headaches. Henna seeds are used to cure dysentery and diarrhoea. The drug is also found in facial and hair lotions, particularly helpful in the treatment of dandruff. It is regarded as abortificient in African traditional medicine [5]. Pharmacological studies have shown that Lawsonia inermis exhibits a wide range of therapeutic effects, including antimicrobial [17]-[22], antioxidant [23]-[27], antidiabetic [28]-[31], antimalarial [32]-[33], abortificient [34], wound healing [35]-[38], anticancer [39]-[42], anti-inflammatory [42]-[47], and other health benefits. This review will examine the chemical constituents, toxicity, and varied therapeutic potential of *Lawsonia inermis*.

II. COMMAN NAMES

Sanskrit: Mehndi, Mehndika, Raktagarba, Kuravaka; English: Henna, Samphire; French: Henne; Hindi: Hena, Mehndi; Gujarathi, Dukhini, Mahrathi; Punjabi: Mehndi, Panwar; Bengali: Mehedi, Mendi, Shudi; Kashmiri: Mohouz; Persian: Hina; Arabic: Yoranna; Sinhalese: Meritondi; Burmese: Dambin; Tamil: Maruthonri, Aivanam, Marithondi; Telegu: Goeranta, Kuravamu; Malayalam: Mailanchi; Canarese: Madarangi; Konkani: Methhi, Padche-methi; Malayese: Hinie, Pontaletsche[1].
Other names:Alcanna, Egyptian pivet, Jamaica Mignoette, Mignonette Tree, Raseda, Henne, Mehndi, Mendee, Smooth Lawsonia[5].

III. SYNONYMS

Homotypic Synonyms- Lawsoniaalba Lam. Heterotypic Synonyms- Lasoniainermis var. spinosa (L.) Pers., Lawsoniaspinosa L., Alkannaspinosa (L.)Gaertn.,CasearíamulifloraSpreng., Lawsonia alba var. flavescensHassk., Lawsoniacoccinea Sm., Lawsoniaflacifolia stokes, Lawsoniapurpurea Lam., Rotanthacombretoides Baker [6].

IV. TAXONOMY

The taxonomical classification of Lawsonia is given below in table.I [7].

Table.I Taxonomical classification.

Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Myrtales
Family	Lythraceae
Genus	Lawsonia L.
Species	Lawsoniainermis L

V. TRADITIONAL USE

Headaches & Hair Care: The juice of the plant with sweet oil, is applied to the head to relieve headaches. Siddha practitioners also use it in special oil formulations to treat gray hair. Burning Sensation in Feet: Fresh leaves, ground into a paste with vinegar or lime juice, are applied to the soles of the feet to relieve burning sensations. Rheumatism & Smallpox: A mixture of leaves, oil, and resin is used to treat headaches and is also applied to the feet during smallpox to protect the eyes. Soft paste of ground leaves or herb with water is used in treatment of Rheumatism. Wounds & Ulcers: An ointment made from the leaves is used to treat wounds and ulcers, while a decoction is applied externally for bruises, sprains, burns, and inflammations. Gonorrhea &Spermatorrhoea: A decoction of the leaves is used as an injection for gonorrhea, and a mixture of leaf juice, water, sugar, or milk is used to treat spermatorrhoea and hot and cold fits.Liver& Skin Conditions: The bark, when infused, is used to treat jaundice, liver and spleen enlargement, and skin diseases such as leprosy. Hair Dye: The leaves of Lawsonia are used as a natural hair dye, promoting healthy hair growth.Nail& Skin Dye: The dye extracted from the leaves is applied to the hands and nails to protect them from decay and disease. Fragrance & Cosmetics: The fragrant water distilled from the flowers was historically used in baths, oils, and ointments for anointing and embalming, especially by the Jews. Menstrual Disorders: The leaves and seeds are used to treat conditions like menorrhagia (heavy menstrual bleeding), vaginal discharges, and leucorrhoea. A powder made from the leaves and seeds is placed in a cloth bag and used vaginally[1].

VI. MORPHOLOGICAL DESRIPTION

Lawsoniainermis is a shrub or small tree, typically 2–7 meters tall, with smooth bark and cylindrical branches that are quadrangular when young. The leaves are subsessile, elliptic to obovate or oblanceolate, measuring 12–45 mm long and 5–20 mm wide, with an attenuated shape at the acute ends and a submembranous texture. Its panicles range from 5 to 25 cm in length. The fragrant flowers have a pedicel of 2–3 mm and linear, caducous bracts (0.4–0.7 mm long). The receptacle is 1–1.7 mm long, with ovate-deltoid sepals (2–3 mm long) and kidney-shaped petals (1.5–2.5 mm long), which are either greenish-white or yellowish. The stamens feature a 5 mm long filament and a 3 mm long style. The fruits, 4–8 mm in diameter, are membranous and retain the style and calyx[4]. An illustration of Lawsoniainermis is available in Fig.I [8].

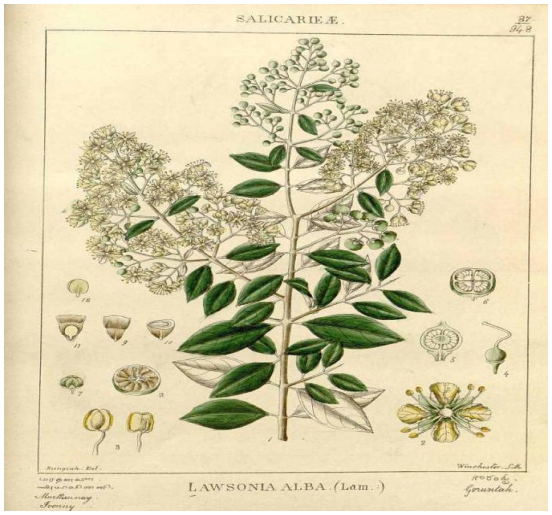
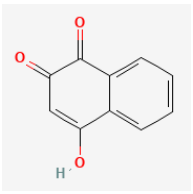


Fig.I Lawsoniainermis

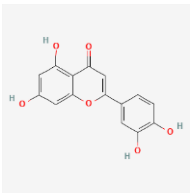
VII. CONSTITUENTS

Leaves of Henna produce 12 to 15 pieces of henna dye [1]. Naphthalene derivatives (1,4-naphthaquinones), specifically lawsone (2-hydroxy-1,4-naphthaquinone), are produced from the precursor 1,2,4-trihydroxy-napthalen-4-beta-D-glucoside when the leaves are dehydrated [5]. It also contains coumarins (laxanthone, I, II, and III), flavonoids,beta-sitosterol-3-O-glucoside, luteolin and its 7-O-glucoside, acacetin-7-O glucoside, , and tannins in all parts [9]. Oil is produced from seeds [1]. Oleic, palmitic, and linoleic acids were identified as the main constituents of the seed oil, with lesser concentrations of stearic, lauric, and myristic acids [11]. Fragrant oil or otto is produced by flowers[1]. Stems contain mannitol (0.7%), although the flowers and roots have lesser concentrations [10]. From the stem bark, an aliphatic hydrocarbon known as 3-methylnonacosan-1-ol was extracted. The structure of lawsaritol, a sterol that was isolated from the

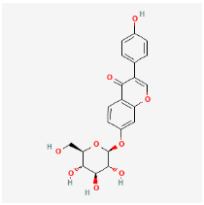
roots, was found to be 24β-ethylcholest-4-en-3β-ol. The structure of isoplumbagin, a naphthoquinone that was extracted from the stem bark, was also determined. Ultimately, lawsaritol A, a dihydroxysterol, was extracted from the roots and its structure was determined [11]. Lawsoniaside and lalioside, two phenolic glycosides, are also present in lawsonia [10].



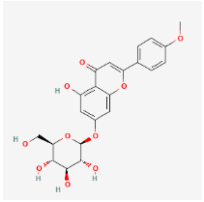
2-hydroxy-1,4-naphthaquinone



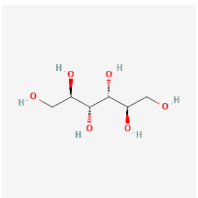
Luteolin



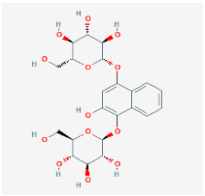
luteolin 7-O-glucoside



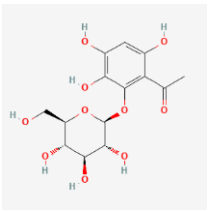
acacetin-7-O-glucoside



Mannitol

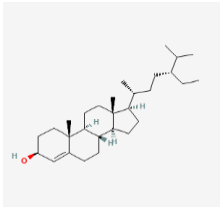


Lawsoniaside

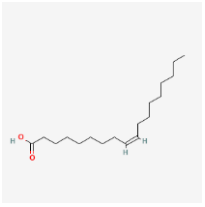


Lalioside

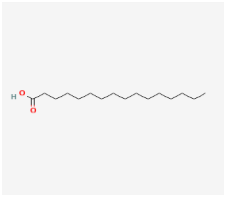
3-methylnonacosan-1-ol



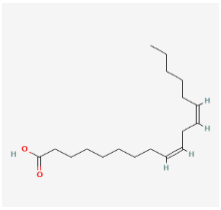
Lawsaritol



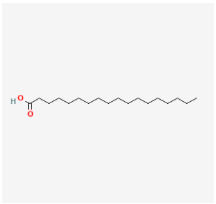
Oleic acid



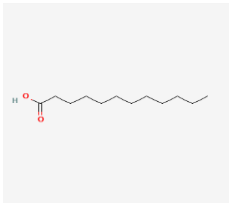
Palmitic acid



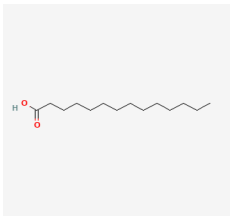
Linoleic acid



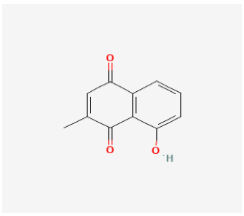
Stearic acid



Lauric acid



Myristic acid



Isoplumbagin

IX. EXTRACTION

A.Soxhlation

Fresh Lawsoniainermis leaves were cleaned and shade-dried for five days before oven-drying at 60°C for five hours.The dried leaves were ground into powder and sealed in a bag before extraction.In a Soxhlet extractor, 350 mL of distilled water and 10 g of powdered leaves were extracted for eight hours.The extract was vacuum-evaporated and stored at 4°C.The leaf extract contained 22.37 ± 0.27 mg/g of lawsone, compared to 24.58 ± 0.11 mg/g in the commercial powder [12]. In a different investigation, 20 g of dried henna leaves were subjected to Soxhlet extraction for eight hours using several solvents, including water, methylene chloride, chloroform, and toluene. The resultant residues gave insufficient IR spectra that did not clearly show Lawsone's functional groups [13].

B. Water bath Extraction

After weighing about 1 g of the material, it was combined with 10 mL of solvent in a 1:10 ratio in a 50 mL conical flask. The flask was sealed and placed in a water bath shaker at 130 rpm. Following extraction, the mixture was filtered, and the filtrate was diluted 80 times before being subjected to an analysis of its total phenolic content (TPC). The TPC of 7203.74 ± 197.8 mg AE/100 g DW, which closely matched the expected value, was obtained from Lawsoniainermis under the ideal extraction conditions of 48.07% acetone, 39.57°C, and 73.78 minutes using Response Surface Methodology (RSM) [14].

B. Maceration

As part of Response Surface Methodology (RSM), the maceration process with a 100% aqueous solvent was selected for extraction and optimized using a central composite design (CCD). A yield of 30.59% was obtained under ideal conditions (40°C, 2 hours, 1:100 ratio) [15]. In a different investigation, 250 mL of methanol was used to soak 15 g of dried leaf powder for a week while being shaken occasionally. The extract was separated using solvents with increasing polarity following filtration and evaporation at 40°C. The hexane layer was separated and dried, while the methanol layer was partitioned into ethyl acetate and aqueous methanol fractions. A hot air oven was used to dry both portions. The maximum recovery yield was 70% for the aqueous methanol extract, while the highest yield was 17% for the crude methanol extract [16]. In a 5-liter Erlenmeyer flask, 80 g of dried henna leaf powder and 4000 mL of distilled water were combined for the maceration procedure. The mixture was then heated to 75°C for six hours, and NaHCO3 was added. Diethyl ether was used to extract the suspension after it had been filtered and acidified to pH 3. A reddish solid (1.1 g) was obtained by combining, washing, drying, and evaporating the ether phases. TLC and IR spectroscopy were used to track Lawsone's isolation. TLC analysis of the fractions produced by column chromatography using a mixture of EtOH:EtOAc (1:2 v/v) revealed that fractions 25–40 had comparable Rf values; however, the ultimate yield was only 30 mg [13].

X. THERAPEUTIC POTENTIAL

A. Antimicrobial activity

Antimicrobial effect of Henna leaf extracts was more pronounced against gram-positive as opposed to gram –negative bacteria, with DMSO, ethanol, and ethyl acetate extracts showing moderate effects. The active compounds were likely lipophilic, with naphthoquinone derivatives, especially Lawsone, being responsible for the observed antibacterial properties. Aqueous, chloroform and di-ethyl ether extracts showed no activity [17]. Extract of dried *Henna* leaves showed antimicrobial activity against both gram-positive and gram-negative bacteria, with chloroform extract being the most efficient in its effect [18]. The ethanolic extract of *L. inermis* (henna) exhibited antimicrobial effect against various pathogens, such as *Candida albicans*, *Echerichia coli* and *Staphylococcus aureus*, and, primarily due to the lawsone molecule. Additionally, phenolic acids and flavonoids like quercetin and rutin enhance its antimicrobial effects [19]. This study found that the aqueous extract of *Lawsonia inermis* was more effective than its alcoholic extract in inhibiting bacteria like *Staphylococcus aureus* and *Pseudomonas aeruginosa*, with active compounds such as gallic acid and lawsone responsible for the antimicrobial effect. Both extracts were ineffective against *E. coli*, and the plant's compounds also showcased potential immunostimulant and antioxidant properties [20]. Another study assessed the antimicrobial activity of *Lawsonia inermis* extracts in inhibiting various bacterial and fungal strains. The aqueous extract showed the strongest effect, particularly against *Staphylococcus aureus* and *Epidermophyton floccosum*, with the lowest MIC of 125 µg/ml, while its extract in petroleum ether had the highest MIC at 500 µg/ml [21]. The aqueous extract of Lawsonia inermis exhibited the highest antimicrobial activity against *S. aureus* and *Epidermophyton floccosum*, with moderate minimum inhibitory concentrations (125 µg/ml) for aqueous and ethanolic extracts, while its extract in petroleum ether had a higher MIC of 500 µg/ml for most isolates [22].

B. Antioxidant activity

The antibacterial and antioxidant qualities of aqueous extract from three henna ecotypes—Shahdad, Roodbar, and Bam—were examined in this work. The extracts inhibited bacterial growth in a dose-dependent manner. Shahdad had the strongest antioxidant activity [23]. Butanolic fraction of lawsonia inermis leaves demonstrated significant antioxidant properties, particularly in hydroxyl radical scavenging, where it outperformed rutin (IC50 = 149.12 µg/mL). The Ferric Reducing Antioxidant power (FRAP) assay, however, showed that its reducing power and capacity to suppress lipid peroxidation were weaker and less effective than trolox. Its activity is dose-dependent, varying across different assays [24]. The hydroalcoholic extract of *Lawsoniainermis* showed 23.66% antioxidant activity but significantly reduced embryo height and weight in mice at both 10 mg/kg and 100 mg/kg doses. Potential developmental concerns were highlighted [25].This study found that the extract of *Lawsoniainermis* flowers in methanol exhibited strong antioxidant activity, with IC50 values comparable to vitamin C in both DPPH

and ABTS assays [26]. This study examined the protective and antioxidant properties of five medicinal herbs against lipid peroxidation in the liver and brain tissues of mice. Strong free radical scavenging activity was demonstrated by the aqueous extracts of Lawsonia inermis, Syzygium aromaticum, Rheum emodi which markedly reduced oxidative damage.Conversely, Picrorhiza kurroa, and Curcuma longa showed reduced antioxidant qualities, underscoring the medicinal potential of particular plants in treating illnesses linked to oxidative stress [27].

C. Anti diabetic activity

This study evaluated the antidiabetic effects of *L. inermis* leaf fractions (A-G) on diabetic mice induced by alloxan. Fractions B, C, and D significantly reduced blood glucose levels, with various bioactive compounds such as flavonoids, alkaloids, and triterpenes likely contributing through mechanisms like insulin-mimetic activity, enzyme inhibition, and oxidative stress reduction[28]. This study found that *Lawsoniainermis* (henna) leaf extracts, particularly methanol fraction C, demonstrated strong antidiabetic effects in diabetic rats[29].The study examined the effects of *Lawsoniainermis*hydroalcoholic extract on alloxan-induced diabetic dyslipidemia in rats. Similar to glibenclamide and metformin, the extract decreased blood glucose by 39.08% at a dose of 400 mg/kg. It also improved total plasma protein, plasma albumin, lipid profile, and serum creatinine [30]. An in vitro antidiabetic assay of petroleum ether (pef) and ethyl acetate fractions (eaf) derived from the crude ethanolic extract of *Lawsoniainermis* leaves revealed the petroleum ether fraction's significant ability to inhibit alpha-amylase activity compared to standard Acarbose. PEF, EAF, and acarbose have corresponding IC50 values of 206.11, 323.1, and 57.2 µg/ml [31].

D. Antimalerial activity

The combination of *Lawsonia inermis* and *Tithonia diversifolia* (1:1) extracts showed strong synergy against *Plasmodium falciparum* in vitro, with significant chemosuppression in P*lasmodium berghei*-infected mice. In contrast, combining *Tithonia diversifolia* with *Lawsonia inermis* and *Chromolaena odorata* was antagonistic in vitro but showed considerable synergism in vivo [32].Treatment with *Lawsoniainermis* at 100 and 200 mg/kg significantly reduced *P. berghei* infection in parasitized mice, while a combination with *Alstoniaboonei* at 50 mg/kg also suppressed the infection and improved packed cell volume. *Lawsoniainermis* at 100 mg/kg elevated liver and kidney enzyme activities (ALT, AST, ALP) but had no effect on serum enzyme levels [33].

E. Abortificient

The study examined the abortifacient effect of *Lawsoniainermis* extract in pregnant mice, with intraperitoneal injections of 1 and 10 mg/kg bw from days 1 to 17 of pregnancy. The treated groups experienced more abortions, with significantly higher estrogen and lower progesterone levels[34].

F.Wound healing activity

According to this study, formulations containing L. inermis boosted the proportion of contractions and dramatically decreased the time it took for burns to heal when compared to control groups. The surface area of the burns treated with various formulations decreased, with the Tazarine type (AQ-LI3) exhibiting the largest drop [35]. This study showed that an ointment made from powder of Henna leaves improved healing of wounds in an excisional model, with better wound contraction and epithelialization than the control group. Histological analysis after 24 days revealed nearly healed skin [36]. According to this study, applying 1% henna ointment helped individuals with epidermolysis bullosa with their skin symptoms, such as redness, itching, burning, and warmth, although the reduction in pain was not statistically significant [37]. This study examined the wound healing effects of Lawsone and its zinc and copper complexes in rats using excision and incision models. The treatments significantly improved wound contraction, reduced epithelialization time, and enhanced antioxidant activity [38].

G. Anticancer activity

Substances Lawsone and its synthetic derivatives, including naphtha [2,3-b]furan-2,4,9(3H)-trione, naphtha[2,3-b]furan-3,4,9(2H)-trione, and 2-(3-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)acetic acid were observed to be the most efficient compounds in demonstrating cytotoxicity against Ehrlich ascites carcinoma (EAC) cells, showing a dose-dependent increase in cell death. [39]. An MTT test was used to evaluate the anticancer activity of L. inermis flower extracts against

HCT116 cells. With an IC50 of 21 mg/L, the chloroform extract showed better anticancer effects than the methanol extract, which had a lower IC50 of 50 mg/L [26]. Studies conducted in-vitro revealed that Ethanolic extract from henna leaves had a notable inhibitory effect against human alveolar basal epithelial, hepatic cancer, and colon cancer cell lines [40]. Given that the mean MI and SCE values were comparable to those of the control group, the Lawsoniainermis extracts (500 µg/ml and 1000 µg/ml) in this study did not exhibit any discernible cytotoxic or genotoxic effects in cultured blood lymphocytes [41]. This study investigated the effects of plumbagin, on B16F10 murine melanoma cells, finding that it reduced adhesion, cell viability, invasion , and migration [42].

H. Anti-inflammatory activity

Methanol extract of *Henna bark* showcased significant and sustained inhibition of paw edema, with reductions of 54.97% and 65.56%, respectively, at the 4-hour mark, compared to 74.17% inhibition by the standard indomethacin [43]. The in vitro anti-inflammatory activity of petroleum ether fraction and ethyl acetate fraction from the crude ethanolic extract of *Lawsoniainermis* leaves was evaluated by testing their ability to inhibit heat-induced and hypotonic solution-induced hemolysis of human erythrocytes. In the heat-induced hemolysis assay, both pulsed electric field(PEF) and ethyl acetate fraction (EAF) showed concentration-dependent anti-inflammatory activity, with EAF exhibiting stronger effects than PEF, closely approaching the standard. Similarly, in the hypotonic solution assay, EAF demonstrated greater activity than PEF, with its effect resembling that of the standard Aspirin [44]. The anti-inflammatory qualities of the test compounds were evaluated in Wistar rats using the carrageenan-induced rat paw edema paradigm. The results showcased that the methanol crude extract and isolated flavonoids showed varying degrees of anti-inflammatory activity. 5, 7, 4'-trihydroxy-6, 3', 5'-trimethoxyflavone (200 mg/kg) showed the best anti-inflammatory activity amongst other isolated flavonoids [45]. The N-butanol and ethyl acetate extracts of *L. inermis* leaves exhibited mild anti-inflammatory action on carrageenan-induced edema in chick paws, with results compared to diclofenac. The effects were concentration- and time-dependent, reaching peak activity at a dose of 50 mg/kg body weight [46]. The study found that the methanol extract of lawsoniainermis flowers exhibited the strongest anti-inflammatory activity, with 87.7% inhibition at 200 mg/L and an IC50 of 49.3 ± 2.3 mg/L. In contrast, the chloroform and n-hexane extracts showed lower activities, with inhibition rates of 31.85% and 26.25%, respectively, at the same concentration. [26]. This study evaluated the anti-inflammatory effect of Lawsoniainermis leaf extract by its ability to inhibit egg albumin denaturation, showing concentration-dependent activity between 4 mg/mL and 10 mg/mL.Diclofenac sodium, used as a reference, showed a stronger effect, with IC50 values of 2 mg/mL compared to 4 mg/mL for the leaf extract [47].

I. Analgesic activity

Mice on treatment with methanolic extract of L. inermis bark at the doses of 300 and 500 mg/kg showed significant ($p<0.05$) decrease in formalin-induced hind paw licking compared to the control group. Furthermore, the 300 mg/kg dose of L. inermis showed stronger action than the conventional medication, diclofenac sodium (10 mg/kg) [48].

J. CNS Depressant activity

Lawsonia inermis (henna) bark methanolic extract was utilized in the study to assess its neuropharmacological effects. The results revealed that the extract exhibited depressive effects on the CNS, as evidenced by the reduction in the exploratory behavior of mice. Additionally, when locomotor activity was assessed, the extract caused a decrease in both the frequency and amplitude of movements, indicating a reduction in motor activity [49].

K. Fibrinolytic and anticoagulant activity

This study assessed the fibrinolytic potential of *Lawsoniainermis* at a 1 mg/ml concentration, demonstrated by increased D-dimer levels and reduced clot weights compared to controls. The findings suggest time-dependent activity, though the exact mechanism and systemic effects require further investigation [50]. This study highlighted the thrombolytic potential of *L. inermis*, *M. nagassarium*, *C. longa*, and *C. sativum*, with the latter two being safe for preventive use due to their established safety as spices. However, further research is needed to address toxicity concerns for *L. inermis* and *M. nagassarium* [51].

XI. TOXICITY

The possible negative effects of Lawsoniainermis leaf extract were evaluated in this study using albino Wistar rats. Doses of 100–2000 mg/kg were used to assess acute toxicity, and then 200–1000 mg/kg were used for 14 days in a sub-acute investigation. Doses of 200 mg & 500 mg/kg showed no discernible negative impact on histological or biochemical measures upon comparison with control group [52]. In this work, Wistar rats were used to evaluate the acute cutaneous toxicity of ethanolic henna leaf extract gel. During the course of the trial, neither body weight nor feed consumption changed significantly, nor were there any signs of aberrant reactions or mortality. Additionally, histopathological analysis revealed no harmful effects on the skin or important organs [53]. This study compared the toxicity of *Rubiatinctorum* and *Lawsoniainermis* plant extracts with conventional insecticides imidacloprid and pirimicarb against *Rhopalosiphumpadi* L. The results showed that imidacloprid was the most effective, while the methanol extract of *Lawsoniainermis* was the least effective [54].

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