PHYTOCHEMICAL PROFILING AND INVITRO PHARMACOLOGICAL STUDIES OF ETHANOLIC *LAWSONIA INERMIS* FLOWER EXTRACT FOR TOPICAL ROLL-ON FORMULATION

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Abstract

Lawsonia inermis L. is a highly branched, smooth shrub or a diminutive tree, grown primarily for its foliage, although parts of the plant including the bark, roots, blossoms, and seeds have been utilized in traditional healing practices. Recent research has shown that an initial phytochemical assessment of the ethanolic extract from Lawsonia inermis flower (MF ET) found the presence of flavonoids, phenolics, proteins, tannins, steroids, and carbohydrates. At a concentration of 100 mg/ml, the ethanolic extract of MF ET displayed a total flavonoid concentration of 57 mg. At a higher concentration of 200 mg/ml, it showed a total phenolic content of 130 mg, which is favorable compared to other levels. The quantitative evaluation of

total flavonoids suggests that the ethanolic extract of MF ET possesses the highest flavonoid concentrations, while phenolic content indicates robust antioxidant potential. The MF ET extract demonstrated notable antioxidant capabilities in the DPPH assay, recording an inhibition rate of 85% at a concentration of 50 mg/ml, with an IC50 value of 45.1232mg/ml, which compares to the standard ascorbic acid's ICs0 of 33.7334 mg/ml. Additionally, evaluating the anti-inflammatory properties using the protein denaturation technique showed that MF ET achieved a 51% inhibition rate at 100 mg/ml, with an IC50 value of 70.7107 mg/ml, in contrast to the standard diclofenac sodium exhibiting an IC50 of 121.29 mg/ml. Thus, shows a moderate level of anti-inflammatory activity compared to diclofenac sodium, while overall; it reveals significant antioxidant capability along with moderate effects on inflammation.

Keywords: Lawsonia inermis flower extract , antioxidant, DPPH assays, Phytochemical, Total Flavonoids, Total Phenol and anti-inflammatory.

INTRODUCTION

Lawsonia inermis, belonging to the Lythraceae family, includes various components such as carbohydrates, phenolic compounds, flavonoids, saponins, proteins, alkaloids, terpenoids, quinones, coumarins, xanthones, fats, resins, and tannins. Additionally, it features 2-hydroxy-1,4- naphthoquinone, commonly known as lawsone. Numerous alkaloids, derivatives of naphthoquinone, phenolic substances, and flavonoids have been extracted from various sections of Lawsonia inermis (Al-Snafi, A.E., 2019). The extracts of ethyl acetate and ethanol derived from the fruit and flowers of L. inermis are believed to be more effective sources of antibacterial compounds in comparison to the extracts from the leaves using the same solvents (Jeyaseelan, E.C., et al 2012). The quantity of volatiles obtained was greater from the yellow flowers, which also exhibited significant differences in volatile profiles compared to the red flowers, with a higher yield of ß-ionone from the former (Wong, K.C. et al 1995). This plant is noted to possess carbohydrates, proteins, flavonoids, tannins, phenolic substances, alkaloids, terpenoids, quinones,coumarins, xanthones, and fatty acids. Reports indicate that the plant exhibits a range of effects including analgesic, hypoglycemic, protective effects on the liver, boosts to the immune system, anti-inflammatory properties, antibacterial and antimicrobial activity, antifungal and antiviral effects, antiparasitic capabilities, efficacy against trypanosomiasis,

antidermatophytic functions, antioxidant properties, interference with fertility, tuberculostatic activity, and anticancer effects (Chaudhary, G., et al 2010). Contemporary pharmacological studies into Henna and its components have validated its abilities to reduce inflammation, lower fever, and provide pain relief(Prosen et al., 2005). Investigations into the phytochemicals present in L. inermis leaves have primarily identified phenolic compounds, such as derivatives of coumarins, flavonoids, naphthalene, and gallic acid, which may undergo glycosylation (Hsouna et al., 2011). Additionally, other substances like triterpenoids, steroids, and aliphatic hydrocarbons have also been extracted from the leaves of this plant (Siddiqui & Kardar, 2001). Phytochemical analysis alongside assessments of cytotoxic and anti-inflammatory properties underscores the leaves of the plant's potential for medicinal applications. The presence of Fraxetin 1(3H) and isobenzofuranone structures was validated in the CHC13 (70%)/MeOH (30%) extract fractions as vigorous active components. Certain extracts derived from the leaves exhibit promising cytotoxic effects on Vero cells. By lowering the chloroform concentration used in the extraction process, there is a corresponding decline in the cytotoxic impact on these cells (Manuja A, et al 2021). The aqueous extract from the Shahdad ecotype showed superior antioxidant capabilities when compared tothose from other ecotypes. The Bam ecotype yielded the greatest concentration of 2-hydroxy-1,4- naphthoquinone, identified as an antibacterial agent. The findings also suggest that additional phytochemical substances may play a role in henna's antibacterial and antioxidant effects (Pasandi Pour A, et al 2020). Extracts from L. inermis, particularly those acquired using polar solvents (HenE and HenM), exhibited significant antioxidant, antibacterial, and anticancer efficacy. Our research emphasizes the beneficial biological characteristics of these extracts, warranting further exploration for their medicinal and industrial use in Thailand (Joyroy N, et al 2025). Lawsonia demonstrated effectiveness in alleviating inflammation in individuals suffering from chronic periodontitis (Takkella BK et al 2023). The pharmacological abilities of Lawsonia inermis can trigger a hypoglycemic response by acting on the alpha-amylase enzyme and can alleviate neurogenic pain (Imam H, et al 2013). Immunohistochemical staining of CD68 in wound images revealed that applying a gelatin-oxidized starch-henna treatment on burn wound sites significantly decreased macrophage counts and inflammatory responses (Hadisi Z, et al 2017). This study focuses on evaluating the in vitro antioxidant properties using the DPPH method and the anti- inflammatory effects through the

protein denaturation technique of Lawsonia inermis flower extract (MF ET) to investigate its phytochemical and pharmacological attributes.

MATERIAL AND METHODS

PHYTOCHEMICAL ANALYSIS TEST

Collection of samples

The plant of the specimen was collected from the agricultural land of Nammakal, Tamil Nadu and the same was authenticated.

Method of preparation of samples

Ten grams of Lawsonia inermis flower extract is heated for five hours in a separate setup with 100 milliliters of ethanol using a reflux condenser in a water bath, then allowed to cool and filtered. The resulting filtrate is evaporated under vacuum to obtain the ethanolic extract. These extract is used for phytochemical and pharmacological properties. Phytochemical screening is the process used to identify various compounds found in plant extracts. Plants contain numerous chemical constituents that can elicit different physiological responses and offer therapeutic advantages. As a result, it is common to assess plants for the presence of phytochemicals that have biological activity and medicinal importance. These constituents are what drive specific biological effects. Examples of these phytoconstituents consist of alkaloids, steroids, carbohydrates, saponins, tannins, flavonoids, and more. (Farnsworth NR. Et al 1966 & Haseen, A., et al 2024).

ESTIMATION OF TOTAL FLAVONOIDS

The concentration of flavonoids in the plant extract was determined through the Aluminium chloride method. A 1 ml sample of Lawsonia inermis flower extract (MF ET) at a concentration of 1 mg/ml in methanol, along with 1 ml of standard quercetin at varying concentrations of 200, 400, 600, 800, and 1000 ug/ml in methanol, were placed into a ten millilitre standard measuring flask. Subsequently, 4 ml of distilled water was introduced, followed by the addition of 0.3 ml of 5% sodium nitrite. After a period of five minutes, 0.3 ml of 10% Aluminium chloride was incorporated. After another five minutes, 2 ml of 1 Molar NaOH was added, and the total volume was adjusted to 10 ml with distilled water. A UV-VIS spectrophotometer was employed to

measure the absorbance of both the crude extract and the standard quercetin at 510 nm while using a reagent blank for comparison. The flavonoid concentration in each gram of dried crude extract reported in milligrams of quercetin equivalents. The absorbance of the test sample was evaluated three times. (MohdNurNasyrig, et al (2014 &A. Muchandi, C. et al 2017).

ESTIMATION OF TOTAL PHENOL

The total concentration of polyphenols within the crude extract was assessed through the Folin-Ciocalteu method alongside a UV-VIS spectrophotometer. Different concentrations of Lawsonia inermis flower extract (Img/ml) in methanol, as well as varying amounts of standard Gallic acid (200, 400, 600, 800, 1000 µg/ml) in methanol, were prepared in separate 25 ml volumetric flasks. Each flask received 9 ml of distilled water, which was thoroughly mixed. To this blend, one millilitre of Folin-Ciocalteu reagent was added and vigorously shaken. After a fiye-minute interval, 10 ml of a 7% Na2CO3 solution was incorporated into the mixture. The total volume was then adjusted to 25 ml using distilled water following a ninety-minute incubation at ambient temperature. Absorbance readings for both the test sample and the standard were recorded at 550 nm, using a reagent blank for reference with a UV-VIS spectrophotometer. The test sample's absorbance was taken three times for accuracy. A linearity curve was established for Gallic acid. (Blainski, A., et al 2013 &Nikolaeva, T.N., et al 2022).

PHARMACOLOGICAL EVALUATION

IN VITRO ANTI OXIDANT ACTIVITY

The ethanolic extract from Lawsonia inermis flowers was examined for its antioxidant properties in vitro through the DPPH method to evaluate its antioxidant capacity. The DPPH method was also applied to test Lawsonia inermis. Antioxidant Activity by DPPH Assay

DPPH (2,2-Diphenyl-1-Picryl-Hydrazyl- Hydrate) Assay

A commonly used method to assess how well antioxidants counteract free radicals is the DPPH test. This technique requires mixing a stable free radical known as DPPH with a plant extract and

subsequently monitoring the color change. A more significant alteration in color indicates increased antioxidant activity. (Baliyan S. et al., 2022).

Principle

The ability of the extracts to counteract free radicals was assessed using the DPPH radical scavenging test, based on the procedures described by Blois and Desmarchelier. The capacity of plant extracts to provide hydrogen atoms was investigated by monitoring the change in color of a methanol solution that includes 2,2-diphenyl-1-picrylhydrazyl. In the presence of antioxidants, the DPPH solution, which starts off as violet or purple, changes to shades of yellow. (Rahman MM et al., 2015).

Procedure

A solution of DPPH with a concentration of 6x10-5M in methanol was prepared by dissolving 7.89mg in 100ml, which means that for a 250ml solution, you would need (7.89/100) x250. After that, 500ul from each sample solution was transferred into an Eppendorf tube. Each concentration was evaluated three times. Next, 500ul of the DPPH solution was added to the sample solution and mixed thoroughly. This combination was then shaken and left at room temperature for thirty minutes. The absorbance was subsequently recorded at 520nm. Ascorbic acid functioned as the positive control, while distilled water served as the negative control. The percentage inhibition in relation to the standard was calculated using the formula below, and the IC_{50} values were also determined. Graph Pad Prism 9 software (Blois MS. 1958 and Karan SK et al., 2012).

Analysis

To analyse data statistically, you need to determine the actual absorbance of the plant material. This involves subtracting the blank plant absorbance from the assay absorbance of the plant. Essentially, after preparing your dilution series, you'll measure the colour intensity of the plant material using a spectrophotometer, which will give you the absorbance reading for the blank plant. For instance, after diluting the plant, it will have a certain colour, and when you measure its colour intensity, you might find the absorbance to be A for the blank plant. If the DPPH assay shows an absorbance of B, then the actual absorbance for the assay would be calculated as B minus A. Therefore, the equation for measuring actual absorbance is: Assay absorbance minus Blank absorbance.

Following that, the percentage inhibition values must be computed using this formula:

% Inhibition = (Absorbance of Blank DPPH solution minus Actual absorbance) multiplied by 100% divided by Absorbance of Blank DPPH solution.

To determine the absorbance of the transparent DPPH solution, you will measure it after preparing the DPPH solution. You will assess its absorbance using a spectrophotometer. By repeating this method for both positive and negative controls, you should create a log-concentration time graph, and the IC_{50} value can be identified by employing a nonlinear regression model with Graph Pad Prism software. Subsequently, by contrasting the experimental results with the controls, you will be able to interpret the data findings.

IN-VITRO ANTI INFLAMMATORY ACTIVITY

Principle of In vitro Egg Albumin Denaturation Method

The most objective of the egg whites denaturation measure is to discover out in case certain operators or compounds can stop or moderate down the method of egg whites getting to be denatured beneath particular conditions. Denaturation alludes to the alter in a protein's structure that leads to a misfortune of its natural work. In this try, egg whites serves as a demonstrate protein, and denaturation happens when it is uncovered to extraordinary warm, changing pH levels, or other denaturing substances. Amide denaturation, the initial shape of egg whites is changed, influencing its physical properties and coming about in a misfortune of its utilitarian movement. The test surveys how well a medicate or compound can avoid or diminish the denaturation of egg whites, which makes a difference assess its anti-inflammatory impacts. The fundamental rule of the egg whites denaturation measure is that substances with anti-inflammatory properties might balance out protein structures and anticipate denaturation, a prepare regularly related with irritation and tissue harm. Hence, operators or compounds that altogether diminish egg whites denaturation in this test might possibly display anti-inflammatory impacts. Protein denaturation is accepted to be one of the components contributing to irritation. Non-steroidal anti-inflammatory drugs (NSAIDs) not as it were avoid protein denaturation but

too restrain the COX enzyme at the same time. Different concentrations of the test can be blended with egg whites arrangement beneath controlled exploratory conditions, permitting the responses to occur sometime recently measuring the absorbance to calculate the rate of restraint (Dharmadeva S et al., 2016).

Inhibition of Albumin Denaturation

The mixture was created by blending 0.5 ml of Lawsonia inermis flower extract with 0.45 ml of a 5% solution of bovine egg whites. The pH level of this mixture was adjusted to 6.3 using a small amount of 0. 1N HCI while maintaining the temperature at 37 °C for 20 minutes. Subsequently, the temperature was raised to 57 °C for a duration of 30 minutes. After cooling, the mixture was transferred to 96-well plates, and absorbance was assessed at 660 nm. The standard used was Diclofenac sodium at a concentration of 1000ug/ml, while the control consisted of 0.05 ml of distilled water. The percentage of inhibition of egg white denaturation was determined using the following formula,

$$\text{Percentage of Inhibition (\%)} = \frac{\text{Absorbance of Control - Absorbance of Test Sample}}{\text{Absorbance of Control}} \times 100$$

Where, Control - Mixture of reagents excluding the medication, Test Sample - Mixture of reagents that includes the sample.

Formulation of Anti-Inflammatory Roll-On

Materials

The anti-inflammatory roll-on was developed using naturally derived ingredients with good dermatological compatibility. Aloe vera gel was selected as the primary base because of its inherent gelling property, calming effect on the skin, and high biocompatibility. Glycerin was incorporated to enhance skin hydration, while distilled water was employed as the dispersion medium. The floral extract of *Lawsonia inermis* was included as the key bioactive ingredient responsible for anti-inflammatory activity. A small amount of lavender or eucalyptus essential

oil was added to impart a pleasant aroma and to provide a gentle cooling sensation upon application.

Method of Preparation

Preparation of Gel Base

Fresh leaves of *Aloe vera* were thoroughly cleaned with water to remove surface impurities. The outer rind was carefully separated, and the inner clear gel was collected. The obtained gel was mildly homogenized and passed through a filter to eliminate fibrous residues. Glycerin (10% w/v) was then blended uniformly with the gel. Distilled water was added slowly with continuous stirring until a smooth, free-flowing gel appropriate for roll-on use was obtained.

Incorporation of *Lawsonia inermis* Extract

A measured quantity of concentrated *L. inermis* flower extract (2–5% w/v) was first dissolved in a small volume of warm distilled water. This solution was gradually introduced into the aloe-glycerin base with gentle mixing to achieve even distribution of the extract. Stirring was continued until a uniformly colored gel was formed, indicating proper dispersion of the phytochemical constituents.

Addition of Fragrance

For improved consumer acceptability, 2–3 drops of lavender or eucalyptus essential oil were added to the formulation and mixed carefully. In addition to fragrance, these essential oils contributed mild soothing and cooling effects.

Final Adjustment and Evaluation

The prepared gel was examined for uniformity, smooth texture, and the absence of particulate matter or entrapped air. The viscosity was adjusted, if necessary, by adding either distilled water or aloe vera gel. The pH of the formulation was maintained within the range of 5.5–6.0 to ensure suitability for topical application.

Filling and Storage

The finalized gel was transferred into clean roll-on containers that had been rinsed with ethanol, using a sterile funnel. The roller ball and caps were securely fitted to prevent leakage. The formulation was stored at ambient temperature away from direct light, preferably in amber-colored bottles. When stored under refrigerated conditions, the roll-on remained stable for approximately 2–3 months.

RESULT

The ethanolic extract of Lawsonia inermis flower has demonstrated both anti- inflammatory and antioxidant effects. The ethanolic extract of Lawsonia inermis flower of yield percentage is 2%. A Preliminilarly phytochemical analysis of the ethanolic extract from Lawsonia inermis flower extract has revealed the presence of flavonoids, phenols, tannins, steroids, and carbohydrates The current study reveals that the ethanolic extract of Lawsonia inermis flower extract at a concentration of 100, contains a total flavonoid content of 57 mg, which is favorable compared to other concentrations and the ethanolic extract of Lawsonia inermis flower, at a concentration of 200, contains a total phenol content of 130 mg, which is favorable compared to other concentrations. The quantitative analysis of total flavonoids indicates that the ethanolic extract of has the highest levels of flavonoids and phenol demonstrates that exhibits strong antioxidant activity. At a concentration of 50 mg/ml, the DPPH assay revealed an inhibition percentage of 85% , with an IC_{50} value of 45.1232mg/ml, in contrast to the standard ascorbic acid, which had an IC_{50} value of 33.7334 mg/ml. This indicates that Lawsonia inermis flower antioxidant activity via the DPPH assay is significant when compared to Vitamin C.To assess anti-inflammatory activity in vitro, the albumin denaturation method was utilized. At 100 mg/ml, Lawsonia inermis flower extract demonstrated a 51% inhibition and an IC_{50} value of 70.7107 mg/ml, compared to the standard diclofenac sodium, which had an IC_{50} value of 121.29 mg/ml . Throughout our investigation of various concentrations in a dose-dependent manner, we found that many concentrations exhibited antioxidant and anti- inflammatory properties of Lawsonia inermis flower extract. When compared to diclofenac sodium, Lawsonia inermis flower extract showed moderate anti-inflammatory effects, indicating it's significant for anti-oxidant activity.

DISCUSSION

The egg albumin denaturation assay is utilized to assess a drug or chemical's ability to prevent or reduce the denaturation of egg albumin, which serves as an indicator of its anti-inflammatory properties, as noted in a prior study (Dharmadeva S et al., 2018). Antioxidant resistances include of distinctive compounds and proteins that work together to neutralize and apportion with responsive oxygen species (ROS). The affiliation between ROS and tumor change is confusing and changes by setting; in any case, afterward ask approximately appears that the neutralization of ROS may development tumor advancement and metastasis in various cancer sorts through a couple of rebellious (Hawk, M.A., et al 2018). A past study indicated that extracts were obtained sequentially utilizing hexane, chloroform, and methanol. The levels of phenolics (ranging from 7.8 to 239.0 grams of gallic acid equivalent per kilogram of dry weight), tannins (between 12.5 and 148.5 milligrams of catechin equivalent per kilogram of dry weight), and flavonoids (from 36.2 to 43.75 milligrams of quercetin equivalent per kilogram of dry weight) were measured. The methanol extract exhibited notable antioxidant properties (IC50 equal to 8.5 mg/L via DPPH assay and IC50 of 5 mg/L through ABTS assay) and demonstrated effective anti-inflammatory capabilities against 5-Lipoxygenase (IC50 of 49.33 mg/L) as reported by Chaibi, R. and colleagues in 2015. Lawsonia inermis, belonging to the Lythraceae family, has a long-standing reputation in traditional medicine for addressing various health issues, including skin disorders, bacterial infections, jaundice, kidney stones, and inflammation. This current research focuses on evaluating the in vitro antioxidant properties and in vivo liver-protective effects of the butanolic fraction (But- LI) derived from the leaves of Lawsonia inermis, as detailed by Kumar M and associates in 2017. In a previous study, it was found that an ethanolic extract of Lawsonia inermis at dosages of 200 mg/kg and 400 mg/kg led to a notable decrease in serum concentrations of pro-inflammatory factors TNF-a, IL-6, and plasma neutrophil counts in rats with croton oil-induced hemorrhoids, as noted by Nallajerla SK and others in 2022. Quantitative assessments of total phenols and total flavonoids reveal a significant quantity of both. The antioxidant and anti-inflammatory properties of medicinal plants (Krishnaiah, D., et al 2011) are attributed to the presence of phenols and flavonoids (Dangles, O., 2012 &Agati, G., et al 2012). Our research also highlights a polyherbal formulation enriched with flavonoids and phenolic content, which may contribute to its antioxidant activity. Furthermore, inquiries regarding the

Lawsonia inermis flower have confirmed its ability to neutralize free radicals, showcasing its antioxidant properties and effectiveness in reducing acute inflammation and pain.

S.NO	PHYTOCHEMICAL TEST	ETHANOLIC EXTRACT
1	Alkaloids	-
2	Flavonoids	+
3	Tannins	+
4	Phenols	+
5	Protein	-
6	Saponin	-
7	Carbohydrates	+
8	Steroids	+
9	Terpenoids	-

Table no 1 : Preliminary Phytochemical studies of *Lawsonia inermis* extract

(+ Presence , - Absence)

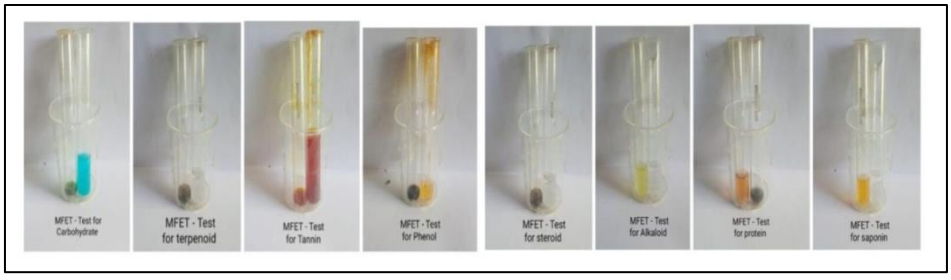

Figure no 1 : Preliminary Phytochemical test of Lawsonia inermis extract

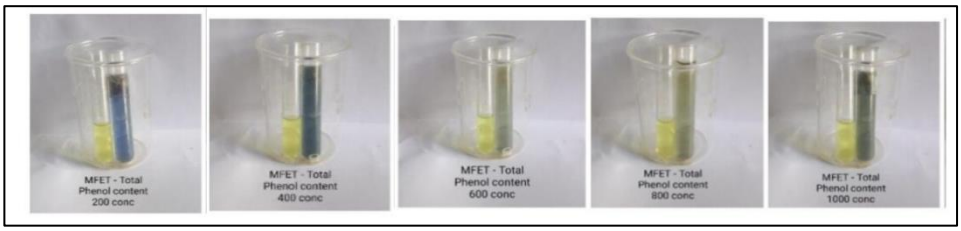

Figure no 2 : Total phenol content of Lawsonia inermis extract

S.NO	Concentration (µg/ml)	Sample	Standard	Total phenol
1	200	0.06	0.10	130mg
2	400	0.08	0.13	107mg
3	600	0.08	0.10	100mg
4	800	0.09	0.10	100mg
5	1000	0.11	0.13	90mg

Table no: 2 Total Phenol content of ***Lawsonia inermis*** Flower extract

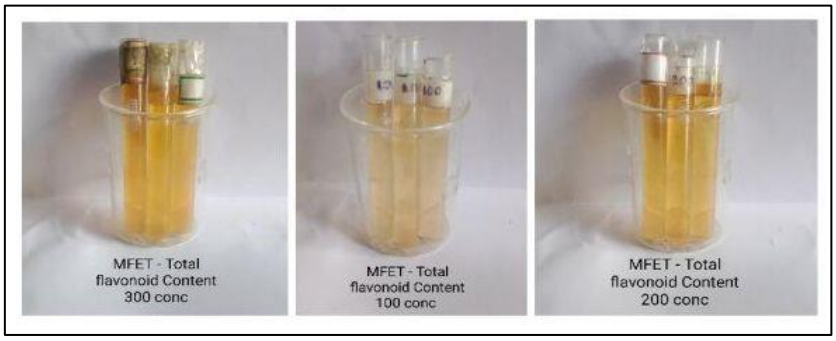

Figure no 3 : Total Flavanoid content of *Lawsonia inermis* extract

S.NO	CONCENTRATION	OD	AVERAGE	TOTAL FLAVANOIDS
1	100	0.06	0.04	**57mg**
2		0.04		
3		0.04		
1	200	0.06	0.05	45mg
2		0.05		
3		0.06		
1	300	0.07	0.06	**50mg**
2		0.06		
3		0.07		
1	**Standard (Quercetin)**	0.48	-	
2		0.58		
3		0.85		

Table no: 3 Total flavanoid content of *Lawsonia inermis* Flower extract

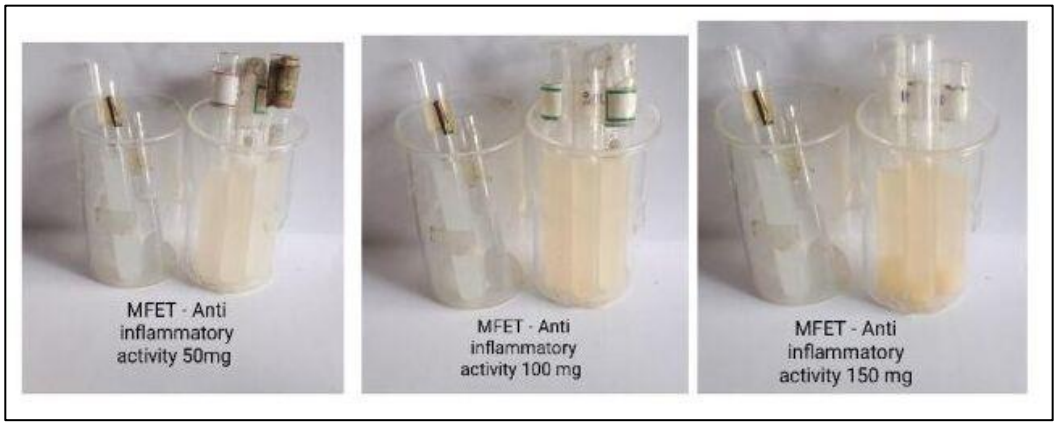

Figure no: 4 Test of anti- inflammatory activity of *Lawsonia inermis* flower extract

S.NO	Concentration(mg)	COD	SOD	%Inhibition	Average(%)	IC_{50} (mg/ml)
1	**50mg**	0.30	0.16	46%	**46%**	**70.7107 mg/ml**
2		0.30	0.16	46%		
3		0.30	0.16	46%		
1	**100mg**	0.30	0.15	50%	**51%**	
2		0.30	0.15	50%		
3		0.30	0.14	53%		
1	**150mg**	0.30	0.22	26%	**28%**	
2		0.30	0.24	20%		
3		0.30	0.18	40%		
In vitro standard Diclofenac sodium						
1	100mg	0.30	0.06	80%	77%	
2		0.30	0.08	73%		
3		0.30	0.06	80%		

Table no: 4 Invitro anti- inflammatory activity of *Lawsonia inermis* flower extract

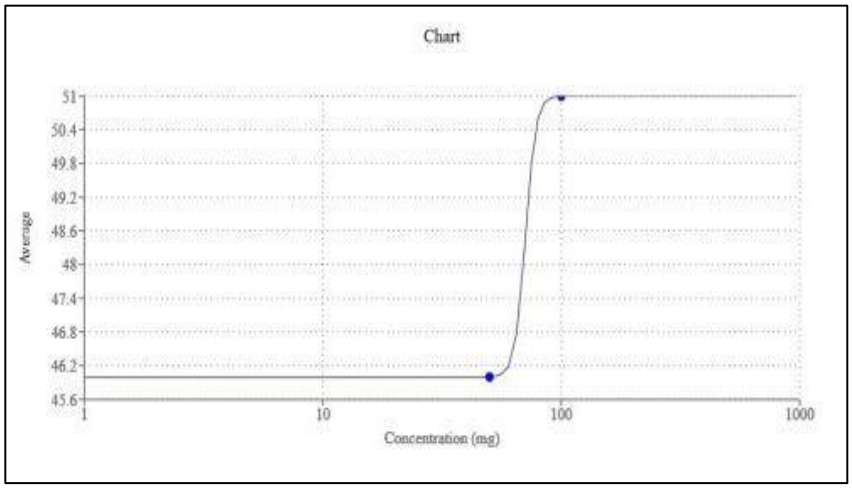

Figure no :5 *Lawsonia inermis* flower extract anti-inflammatory IC_{50} value

S.NO	Concentration(mg)	Average	IC_{50} value
1	50	46%	**70.7107mg/ml**
2	100	51%	
3	150	28%	
Standard Diclofenac sodium			
S.NO	Concentration(mg)	Average	IC_{50} Value
1	50mg	91%	121.29mg/ml
2	100mg	93%	
3	150mg	85%	
4	200mg	89%	
5	250mg	88%	

Table No:5 *Lawsonia inermis* flower extract anti-inflammatory IC_{50} value compared to standard Diclofenac sodium

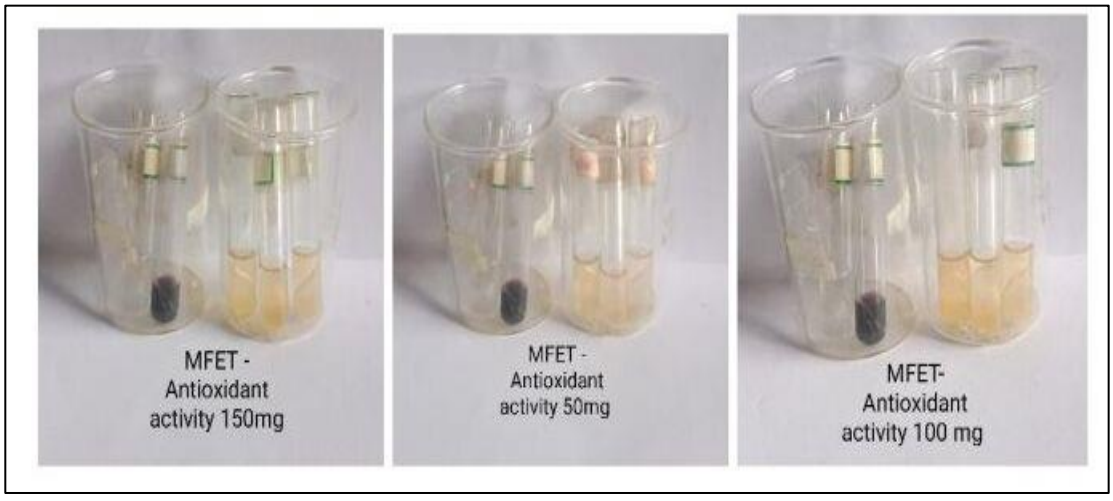

Figure no: 6 Test of anti-oxidant activity of *Lawsonia inermis* flower extract

S.NO	Concentration(mg)	COD	SOD	% Inhibition	Average(%)	IC_{50} (mg/ml)
1	**50mg**	0.30	0.05	83%	**85%**	**45.1232 mg/ml**
2		0.30	0.05	83%		
3		0.30	0.03	76%		
1	**100mg**	0.30	0.08	73%	**77%**	
2		0.30	0.05	83%		
3		0.30	0.07	76%		
1	**150mg**	0.30	0.05	83%	**83%**	
2		0.30	0.05	83%		
3		0.30	0.05	83%		
In vitro standard Vitamin C						
1	100mg	0.30	0.02	93%	93%	
2		0.30	0.02	93%		
3		0.30	0.02	93%		

Table No: 6 *Lawsonia inermis* flower extract In vitro antioxidants activity by DPPH assay

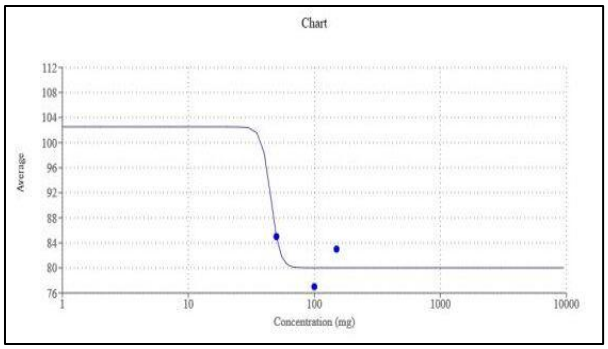

Figure no :7 *Lawsonia inermis* flower extract anti-oxidant IC_{50} value

S.NO	Concentration(mg)	Average	IC_{50} value
1	50	85%	**45.1232mg/ml**
2	100	77%	
3	150	83%	
Standard Vitamin C			
S.NO	Concentration(mg)	Average	IC_{50} Value
1	50mg	91%	33.7334 mg/ml
2	100mg	87%	
3	150mg	86%	
4	200mg	92%	
5	250mg	84%	

Table No:7 *Lawsonia inermis* flower extract antioxidant IC_{50} value compared to standard vitaminC $IC_{50\ value}$

SUMMARY AND CONCLUSION

This study investigated the pharmacognostical, physicochemical, phytochemical, antioxidant, and anti-inflammatory properties of *Lawsonia inermis* Linn. flowers to validate their traditional medicinal use. The plant material was authenticated and processed using standard methods, and pharmacognostical evaluation confirmed the characteristic features of the flowers. Physicochemical parameters, including extractive values, ash values, foreign organic matter, and moisture content, were within permissible limits, indicating good quality and purity. The ethanolic extract showed the highest extractive value, suggesting efficient extraction of polar bioactive compounds. Phytochemical screening revealed the presence of flavonoids, phenols, tannins, carbohydrates, and steroids, with quantitative analysis showing appreciable total phenolic and flavonoid contents. These compounds are known to play a major role in antioxidant

and anti-inflammatory activities. The extract exhibited dose-dependent inhibition of protein denaturation, confirming its in vitro anti-inflammatory potential. Significant free radical scavenging activity was also observed in the DPPH assay, comparable to standard antioxidants. The biological activities are closely associated with phenolic and flavonoid constituents present in the extract. Overall, the findings support the traditional use of *Lawsonia inermis* flowers and indicate their potential application in herbal formulations for managing oxidative stress and inflammation.

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