Phytochemical screening and in-vitro evaluation of antioxidant activity of methanolic extract of lawsonia *inermis leaves*

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ABSTRACT

INTRODUCTION

Antioxidants have an important role in health, to prevent oxidative stress or oxidation reaction in the body that can lead to cardiovascular disease, cancer, and aging. The present study investigates the phytochemical composition and antioxidant potential in *Lawsonia inermis*. *Lawsonia inermis* is rich in phytochemicals like alkaloids, saponins, steroids, flavonoids and glycosides. It is used in diabetes, Jaundice, leprosy and fungal infection.

MATERIAL AND METHODS

Standard qualitative tests were performed to detect the presence of alkaloids (Mayer's, Wagner's reagent), flavonoids (Shinoda test), tannins (Ferric chloride test), saponins (froth test), terpenoids (Salkowski test), phenols, and glycosides.The phenol content was determined by using Folin-Ciocalteu method using gallic acid and flavonoids content was determined by using aluminum chloride colorimetry method assay. While antioxidant activity was carried out by using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and ascorbic acid in UV Spectrophotometer.

RESULTS

Phytoconstituents like alkaloids, tannins, coumarins, terpenoids, phenolic compounds and flavonoids were present while carbohydrate was absent. Total phenolic content in the methanolic leaves extract was found to be highest at 43.56 mgGAE/gm and lowest was found to be 12.89 mgGAE/gm. Total flavonoid content in the methanolic extract was found to be highest 81.28 mgQAE/gm and lowest was found to be 9.23 mg QAE/gm. The methanolic extract of *Lawsonia inermis* by DPPH Scavenging method with the highest inhibition percentage was found to be 83.86 ug/ml and lowest percentage of inhibition was found to be 49.70 ug/ml respectively.

CONCLUSION

From the result, it can be concluded that the methanolic extract of *Lawsonia inermis* contains antioxidant properties with high percentage of free radical scavenging activity. The obtained bioactive compounds should be standardized and developed to reduce oxidative stress related disease and improve the physiological function of the human body.

KEYWORDS

Lawsonia inermis, Free radicals, Antioxidant, Ascorbic acid, DPPH

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INTRODUCTION

Reactive oxygen species (ROS) such as superoxide anions and hydroxyl radicals are continuously generated in the human body during metabolic processes. When their production exceeds the antioxidant defence capacity, oxidative stress occurs, contributing to chronic diseases including diabetes, cancer, cardiovascular disorders, and neurodegenerative conditions.[1] Natural antioxidants from medicinal plants have gained increasing interest because synthetic antioxidants like Butylated hydroxyanisole (BHA) and Butylated Hydroxytoluene (BHT) may pose health risks when used long-term.[2] Medicinal plants contain diverse phytochemical constituents such as phenolics, flavonoids, alkaloids, tannins, and terpenoids that play a key role in free-radical scavenging and antioxidant activity.[3] Lawsonia inermis L. (henna), widely used in traditional medicine across tropical and subtropical regions, is known for its therapeutic properties against inflammation, skin disorders, microbial infections, and liver ailments. It is a biennial dicotyledonous herbaceous shrub. A native of North Africa and South-West Asia, the plant is now widely cultivated throughout the tropics as an ornamental and dye plant. A much-branched glabrous shrub or small tree (2 to 6 m in height). Leaves are small, opposite in arrangement along the branches, sub-sessile, about 1.5 to 5 cm long, 0.5 to 2 cm wide, greenish brown to dull green, elliptic to broadly lanceolate with entire margin, petiole short and glabrous and acute or obtuse apex with tapering base. Young branches are green in colour and quadrangular which turn red with age. Bark is greyish brown, unarmed when young but branches of older trees are spine tipped. Inflorescence is a large pyramid shaped cyme. Flowers are small, about 1 cm across, numerous, fragrant, white or rose coloured with four crumbled petals. Calyx is with a 0.2 cm tube and 0.3 cm spread lobes. Fruit is a small brown coloured round capsule. Fruit opens irregularly and splits into four sections at maturity and is many seeded. Seeds are about 3 mm across, numerous, smooth, pyramidal, hard and thick seed coat with brownish coloration.[4] Its pharmacological activities are largely attributed to bioactive compounds such as lawsone, flavonoids, tannins, and phenolic acids.[5] Given the increasing interest in plant-derived antioxidants and the ethnomedicinal value of *L. inermis*, scientific evaluation of its phytochemical constituents and antioxidant potential is essential. Therefore, this study aims to investigate the phytochemical profile and in-vitro antioxidant activity of the methanolic extract of *Lawsonia inermis* leaves.

MATERIAL AND METHODS

The present study was carried out from November 2023 to May 2024 in the department of Pharmacy. Universal College of Medical Sciences and Teaching Hospital, Bharahawa, Nepal, after taking approval from Institutional Review Committee with IRC No: UCMS/IRC/077/23.

Collection and authentication of plant material

Plant materials were collected from Siddharthanagar Municipality, Rupandehi district of Nepal. Herbarium was prepared with fresh plant material and was submitted for identification and certification of plant. Certificate was issued by Assistant professor Pushpa Raj Poudel, Department of Horticulture and Plant protection, Institute of Agriculture and Animal Science, Paklihawa Campus, Bhairahawa

Extraction

The leaves of Lawsonia inermis were washed, shade-dried and grounded into a coarse powder and stored in airtight container. Hot extract was prepared by using Soxhlet extraction method. About 50 g powder was extracted with 400 ml of solvent (methanol) at 40-60 ^{0}C for 24 hours. The extract was stored in the refrigerator at 4 ^{0}C for further study use.[6-8]

Phytochemical screening

Preliminary phytochemical screening of methanolic leaves extract was done using the standard procedure.[9,10]

Determination of total phenolic content

The total Phenolic content of leaves extract was determined by using Folin-Ciocalteu method. 1 ml 2N Folin Ciocalteu reagent was added to different concentration of plant extract which were prepared by using stock solution (1000 ug/ml) diluted to 10, 20, 30, 40, and 50 ug/ml with methanol than shaken for 2 min and 2 ml of sodium carbonate (7.5%) was added and the mixture was incubated at 45 ^{0}C for 15 min. The absorbance was read at 765 nm using UV-Visible spectrophotometer. The standard curve of gallic acid was plotted (10 ug/ml to 50 ug/ml) as the standard, and the total phenolic content was expressed as mg gallic acid equivalent per gram of dried sample (mgGAE/g) concentrations.[11,12]

Determination of total flavonoids content

The total flavonoid content of leaves extract was determined by aluminum chloride colorimetry method. 10 mg of plant extract dissolved in 10 ml of methanol of different concentrations (50, 100, 150, 200, and 250) which were prepared from stock solution of 1000 ug/ml) was taken and volume make up to 10 ml diluted with methanol. Then, 0.3 ml of 5% of $NaNO_2$ solution was added to the mixture. After 6 min, 0.2 ml of 10% $AlCl_3$.6 H_2O_2 solution was added to the mixture and solution was allowed to stand for 5 minutes. Subsequently, 1 ml of NaOH was added.[9] The solution was incubated for 30 minutes and absorbance was measured at 510 nm and quercetin as standard also prepared as same sample concentration.

The concentration of flavonoids in test solution was calculated and expressed as equivalent of quercetin (mg QE/g) of sample, the triplicate the test sample was taken and average was determined, using a standard curve generated with quercetin.[12,13]

Antioxidant activity using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay

The free radical scavenging activity of the extract was determined by 2,2-diphenyl -1-picrylhydrazyl assay. The

DPPH radical has a purple color that turns to yellow after reduction by antioxidants in the herbal extracts. The antioxidants scavenging ability of Lawsonia inermis leaves extract (1000 ug/ml) of various concentrations (50,100,150,200 and 250) was added to a methanolic solution. A dilution series of Lawsonia inermis leaves extract was prepared. 1 ml of DPPH reagent prepared in methanol (4 mg/100 ml i.e 0.1mM) was added to test and standard sample of various concentration. The mixture was allowed to stand for 30 min in the dark and absorbance was measured at 517 nm. Ascorbic acid was used as the standard and the methanol was taken as blank. Mixture of 1 ml DPPH and 3 ml methanol solutions were taken as control.[14,15]

Scavenging activity %= 1-(absorbance sample/absorbance control) ×100

Statistical analysis
The in-vitro study was performed in triplicate, and the result of the study was expressed as Mean ± SD. IC50 value was calculated by plotting the data in MS-excel.

RESULTS

Qualitative phytochemical screening
The methanolic extract of L. *inermis* showed the presence of several classes of phytochemicals. Table 1 summarizes the findings.

Table 1. Qualitative phytochemical composition of *L. inermis* leaf extract

S. N.	Phytochemicals	Test	Results
1.a		Mayer's test	+
b.	Alkaloids	Wagner's test	+
c.		Hager's test	+
2.	Tannin	Braymer's test	+
3.	Terpenoids	Salkowski test	+
4.	Saponins	Saponin test	+
5.	Steroids	Steroids test	+
6.	Flavonoids	Ferric chloride test	+
7.	Phenol	Ferric chloride test	+
8.	Coumarins	NaOH test	+
9.	Carbohydrate	Molisch's test	-

(+ = Present, – = Absent)

Quantitative phytochemical screening
total phenolic Content (TPC)
The methanolic extract of Lawsonia inermis was evaluated at five different concentrations 10,20,30,40 and 50 µg/mL. The TPC values increased in a concentration-dependent manner. Among the tested concentrations, the highest TPC was recorded at 50 µg/mL, with a value of 43.56 mg GAE/g, while the lowest TPC was observed at 10 µg/mL, giving 12.89mg GAE/g. The value was calculated from the gallic acid calibration curve (y = 0.005x +0.608, R2=0.943) The gradual rise in phenolic content across concentrations indicates efficient solubilization of phenolic compounds contributing to greater antioxidant potential. The results are summarized in Table 2.

Table 2. Total phenolic content of methanolic extract of *L. inermis* leaves at different concentrations

Concentration (ug/ml)	Mean absorbance by methanolic extract	Mean absorbance by gallic acid gallic acid	Total phenolic content (mg of equivalent /gm dry extract) ± S.D
10	0.31	0.64	12.88 ± 0.001
20	0.37	0.74	19.83 ± 0.07
30	0.47	0.76	30.38 ± 0.13
40	0.59	0.82	43.55 ± 0.12
50	0.69	0.85	43.55 ± 0.12

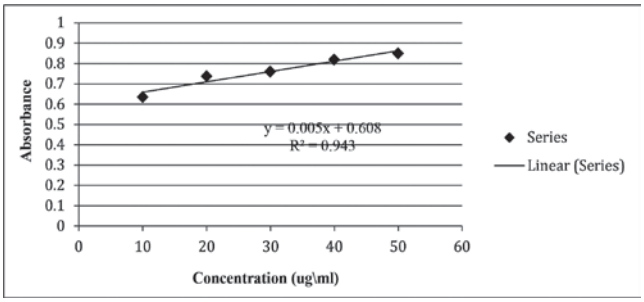

Figure 1. Calibration curve of gallic acid standard used for estimating total phenolic content of the methanolic extract of *L. inermis* leave.

Total flavonoid Content (TFC)
Using the aluminium chloride colorimetric method, the total flavonoid content of the methanolic leaves extract of L. inermis was found to be 313 mg QE/g extract highest at 250 ug\ml and 79 mg QE/g extract lowest at 50 ug\ml. This value was calculated from the standard quercetin calibration curve (y = 0.001x + 0.296, R^2 = 0.998).

Table 3. Total phenolic content of methanolic extract of *L. inermis* leaves at different concentrations

Concentration	Mean absorbance by methanolic extract	Mean absorbance by Quercetin	Total flavonoids content (TFC)
50	0.61	0.37	79 ± 0.01
100	0.57	0.43	118 ± 0.16
150	0.37	0.49	262 ± 0.09
200	0.36	0.56	277 ± 0.09
250	0.41	0.64	313 ± 0.09

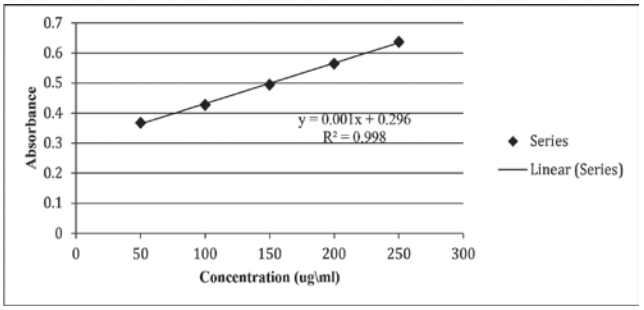

Figure 2. Calibration curve of quercetin used as the standard for measuring total flavonoid content in the methanolic extract of L. inermis

Table 4. DPPH radical scavenging activity of methanolic extract of lawsonia inermis Leaves

S. N	Concentration (ug\ml)	Inhibition percentage of DPPH Scavenging activity of Ascorbic acid	Inhibition percentage of DPPH Scavenging activity of *Lawsonia Inermis*
1.	50	48.61%	19.12%
2.	100	51.38%	24.26%
3.	150	51.74%	36.34%
4.	200	60.05%	43.32%
5.	250	63.02%	60.01%
6.	IC50 value	83.86 ug\ml	49.70 ug\ml

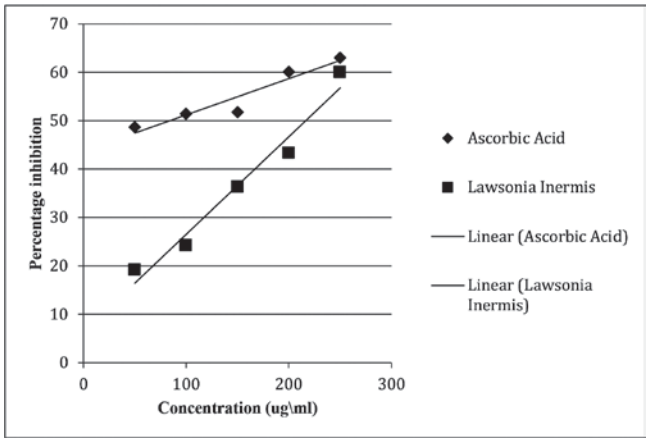

Figure 3. DPPH scavenging activity

X-axis=Concentration of ascorbic acid and methanolic extract of *Lawsonia inermis*
Y-axis=Percentage inhibition of ascorbic acid and methanolic extract of *Lawsonia inermis*

DISCUSSION

The present study investigated the phytochemical composition and antioxidant potential of the methanolic extract of Lawsonia inermis leaves. Phytochemical screening revealed the presence of alkaloids, tannins, coumarins, terpenoids, phenolic compounds, and flavonoids, while carbohydrates were absent. These results partially align with previous studies such as L.C. Chuku et al. (2020)[16] reported alkaloids, tannins, flavonoids, and saponins with absence of carbohydrates, while Hassan R.A. et al. (2013)[17] observed alkaloids and tannins with absence of saponins. Hussaini Majiya et al. (2023)[10] reported the presence of alkaloids, flavonoids, tannins, and carbohydrates. The differences among these studies may reflect variations in plant species, part used, environmental conditions, and extraction procedures.

The total phenolic content (TPC) in this study ranged from 12.89 to 43.56 mg GAE/g, and the total flavonoid content (TFC) ranged from 79 to 313 mg QE/g. Previous studies have reported a wide range of values, for instance, Hassan R.A. et al. (2013)17 found 71.16 mg GAE/g TPC and 32.91 mg QE/g TFC, Issmail Nounah et al. (2017)[11] reported 2.56 mg GAE/g TPC, and Alireza Moulazadeh et al. (2021)[13] reported TPC 96.76 ± 3.34 µg GAE/mg and TFC 197.69 ± 5.76 µg QE/mg. Enayatullah Rahmany et al. (2021)[12] reported TPC as high as 7203.74 mg GAE/100 g. The variations in phenolic and flavonoid content among studies are likely due to differences in plant species and variety, plant part used, maturity, seasonal and climatic conditions, soil composition, and extraction method and solvent.

The antioxidant activity was carried out using the DPPH scavenging method for *lawsonia inermis* leaves, different results were obtained from different concentration of the extract.

Antioxidant activity, assessed by DPPH radical scavenging assay, showed that the methanolic extract had an IC_{50} value of 49.70 µg/mL, while ascorbic acid as a standard showed 83.86 µg/mL whereas Hassan, R. A. et al. (2013)[17] showed that DPPH scavenging activity in the methanolic extract of leaves of *Lawsonia inermis* obtained IC50 value was found to be 23.9 ug/ml and Dina Mostafa Mohammed et al (2022)[18] showed that using two different solvent that the ethanolic extract has lower IC50 (20 ug/ml) and the water extract has higher IC50 value (25 ug/ml). The percentage of inhibition was found to be highest at 63.02 % and lowest was 48.61% in ascorbic acid whereas percentage of inhibition was found to be highest at 49.70 % and lowest to be 19.12 in methanolic extract *Lawsonia inermis* leaves. These results indicate that the methanolic extract of *L. inermis* leaves possesses significant antioxidant activity, likely due to its phenolic and flavonoid content. Differences in IC_{50} values across studies may be influenced by the type of solvent, extraction method, plant part used, and assay conditions. The high antioxidant activity of the methanolic extract can be attributed primarily to its polyphenolic and flavonoid constituents. Phenolic compounds act by donating hydrogen atoms or electrons to free radicals, thereby terminating chain reactions, while flavonoids stabilize radicals through resonance structures and chelation of transition metal ions. The synergistic action of these bioactive compounds likely contributes to the potent antioxidant potential observed in this study.[19,20]

CONCLUSION

The study was based on in-vitro evaluation for observing phytochemical constituents, total phenolic content, total flavonoid content and antioxidant activity of *Lawsonia inermis* leaves by UV Spectrophotometry method. The preliminary phytochemical screening revealed the presence of various secondary metabolites, including alkaloids, flavonoids, phenol, carbohydrates, tannins, and saponins, which are known to possess antioxidant properties. Hence, the findings of this study suggest that *Lawsonia inermis* leaves could be a potential source of natural antioxidants, supporting its use in pharmaceutical, nutraceutical, and functional food applications, and providing a basis for further pharmacological and mechanistic studies. Further studies are recommended to isolate and identify the specific compounds responsible for the antioxidant activity, as well as to evaluate their potential therapeutic applications.

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CONFLICT OF INTEREST
None

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