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Phytochemical Profiling and in Vitro Antioxidant Activity Assessment of Lawsonia Inermis and Juglans Regia Leaf Extracts

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Abstract: *This study aimed to profile the phytochemicals present in Lawsonia inermis and Juglans regia leaf extracts and assess their in vitro antioxidant activity using three different assays. Phytochemical screening revealed the presence of carbohydrates, glycosides, triterpenes, flavonoids, saponins, and alkaloids in both plant extracts. These compounds are known to possess various pharmacological properties, including antimicrobial, anti-inflammatory, antiviral, and anticancer activities. The in vitro antioxidant activity of the extracts was evaluated using the phosphomolybdenum, FeCl3 radical scavenging, and DPPH radical scavenging assays. The results showed that Lawsonia inermis extract had a higher total antioxidant capacity, as measured by the phosphomolybdenum assay, compared to Juglans regia extract. Similarly, it had a higher ability to prevent the formation of free radicals, as measured by the FeCl3 radical scavenging assay. Additionally, Lawsonia inermis extract exhibited a higher capacity to neutralize free radicals, as measured by the DPPH radical scavenging assay. Overall, the study suggests that both Lawsonia inermis and Juglans regia leaf extracts contain various phytochemicals with potential health benefits. However, Lawsonia inermis extract exhibited a stronger antioxidant activity compared to Juglans regia extract. These findings could have significant implications in the development of natural antioxidants for various industrial and therapeutic applications.*

Keywords: Leishmania tropica, Lawsonia inermis, Juglance regia, Anti-leishmania, phytochemistry

I. INTRODUCTION

Leishmaniasis is a worldwide infectious disease caused by parasites of the genus Leishmania, affecting more than 12 million people worldwide. This disease is endemic and causes an increase in the morbidity and mortality in populations of Africa, Asia and Latin America.[1,2]

Plants have served mankind since its inception. In the present scenario, a great interest has been made towards the natural products derived from medicinal plants all over the world as they show to have enormous health care benefits such as antioxidative, antihypertensive, antimutagenic, bronchodilator, skin disorders, antispasmodic, fever, jaundice, anthelmintic, anti-proliferative, anticancer, antimicrobial, antidiabetic, hepatoprotective, larvicidal, antiinflammatory, haemolytic activities etc.[3]

Plants are an important source of bioactive molecules for drug discovery due to the inherent pharmacological activities with low toxicity. India is endowed in the form of medicinal plants having about 45,000 plant species. Recently, around 20,000 medicinal plants have been reported in India, in which a lot of plant species are used since ancient times for curing different diseases. [4]

Currently, the infectious diseases remain the leading cause of death worldwide and infections due to antibiotic resistant ability of some microorganisms. In addition, synthetic antimicrobial agents are often associated with the adverse effects on the host, including immune suppression, hypersensitivity and several allergic responses [5]. This situation reinforced the scientist communities looking for eco-friendly alternatives so that novel bioactive therapeutic agents can be made. [6]

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Lawsonia inermis, commonly known as Mehdi/Mehandi is a shrub or small tree frequently cultivated in India, Pakistan, Egypt, Yemen, Iran and Afghanistan. Henna is an ancient dye, evidence being the Egyptian mummies found in the tombs that had their nails dyed with henna. It is also used in many countries for dyeing hair, eyebrows and fingernails during religious festivals and marriages etc. the powdered leaves of this plant (aqueous paste) are used as a cosmetic for staining hands, palms, hairs and other body parts. [7,8]

Juglans regia L. (Persian walnut) is a deciduous tree from Juglandaceae family. Its fruits are consumed as food, which are rich unsaturated fatty acids. Walnut leaf has been widely used in traditional medicine for the treatment of skin inflammations and ulcers and for its antidiarrheic, anti-helmintic, antiseptic and astringent properties. [9,10]

II. MATERIALS AND METHODS

2.1 Drugs and Chemicals

Amphoterecin b was purchased from Emcure, Mumbai. All the solvents, chemicals used were of analytical grade and chemicals required for sensitive biochemical assays were purchased from Merck. All drug solutions were freshly prepared in saline before each experiment. Lawsonia inermis and Juglace regia extract was dissolved in distilled water and administered orally.

2.2 Plant Material

Leaves of Lawsonia inermis were purchased locally and Juglace regia collected from northen part of India and authenticated by Dr .Rafiuddin Naser , Dept. of Botany, Maulana Azad College of Art Science and Commerce ,Dr. Rafiq Zakaria Campus Aurangabad. A voucher specimen department. no. 12721 has been deposited in the same.

2.3 Extraction

Leaves of Lawsenia inermis were collected and dried under the shade condition, crushed with the help of grinder and stored in the airtight container. The dried crushed leaves were weighed and defatted with petroleum ether (60-80 °C) in Soxhlet's extractor. The marc was dried and again extracted with methanol for 72hrs in Soxhlet's extractor. The aqueous ethanolic extract was evaporated using rotary evaporator. [11,12]

Preliminary Phytochemical Evaluation of Lawsenia Inermis and juglance regia leaves Extract [14,15]

Test of Alkaloids

1. Mayer's Test: Take test solution in the test tube adds the Mayer reagent (Potassium mercuric iodide solution). White or yellow precipitate indicates the presence of alkailoids.
2. Wagner's Test: Take the test solution in a test tube then add Wagner's reagent (iodine solution). Brown or reddish brownprecipitate.

Tests of Glycosides

1. Raymond's Test:- Take the test solution in test tube and add 1 ml of 50% ethanol. Add 0.1% solution of dinitrobenzene in ethanol then added 2-3 drops of 20% sodiumhydroxide solution . Appearance of violet color indicated the presence of Glycosides.
2. Killer Killani Test:- 2 ml of extract in a test tube add glacial acetic acid then add one drop of 5% FeCl3 with conc. H2SO4. Reddish brown color appeared at the junction of the two liquid layers and upper layer appeared bluish green.
3. Legal Test:- Take the test solution in a test tube add few drops of pyridine and a drop of 2% sodium nitroprusside then add a dropof 20% sodium hydroxide solution. Deepred color appears.

Tests for Carbohydrate

1. Molisch's Test:- 2-3 ml. extract add few drops of α- naphthol solution (20% in ethyl alcohol) then 1 ml. conc. H2SO4 addedalong the side of the test tubes. Violet ring was formed at the junction of two liquids.
2. Benedict's Test: To the extract add equal volume of Benedict's reagent. Heat for 5 min. Solution appears green, yellow or red.

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Tests for Tannins

1. Vanillin- HCl Test: To the extract add vanillin-HCl reagent (1 g vanillin + 10 ml. alcohol + 10 ml. conc. HCl). Formation of pink or red color
2. Gelatin Test: To the extract solution add aqueous solution of gelatin. White buff color precipitate are formed

Tests for Flavanoids

1. Lead acetate test: Filter paper strip was dipped in the alcoholic solution of extract,ammoniated with ammonia solution. Color changed from white to orange.
2. Shinoda Test: To the extract add 5 ml. 95% alcohol, few drops of conc. HCl and 0.5 g magnesium turning. Pink color observed.
3. Alkaline Reagent Test: Extracts have to be treated with a few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on the addition of dilute acid, indicates the presence of falvonoids.

Tests for Resins

1. Ferric chloride test: Take the extract in test tube add alcohol with few drops of FeCl3 solution. Green color appears.
2. Turbidity Test: Extract solution (2 g of sample in methanol) add 5 ml distilled water, turbidity appears.

Test for Steroids

1. Libermann- Bur chard Test: To 2 ml. extract add Chloroform, 1- 2ml. acetic acid and 2 drops H_2SO_4 from the side of the test tube. First red, then blue and finally green color appeared.
2. Salkowski Reaction: To 2 ml. of extract add 2 ml. chloroform, 2 ml. conc. H_2SO_4. Shake well. Chloroform layer appeared red color and acid layer shows greenish fluorescence.

Test for Proteins and Amino-acids

1. Biuret Test: Take 3 ml. of extract in a test tube add 4% NaOH and 2-3 drops of 1% copper sulphate solution. Presence ofred/violet coloration.
2. Precipitation test: extract then mix with absolute alcohol. White ppt.
3. Ninhydrin Test: Extract in a test tube then add ninhydrin reagent in boiling water bath for 10 min. Violet color appeared.
4. Cysteine Test: To 1 ml of protein solutionin a test tube, add 2 drops of 10% sodium hydroxide solution and 2 drops of lead acetate. − Mix well and put in a boiling water bath for few minutes; a black deposit
5. is formed with albumin, while a slight black turbidity is obtained with casein due to its lower content of sulfur. Gelatin gives negative result.

Test for Fats

1. Sudan Red test: To a test tube, add equal parts of test sample and water to fill about half full. Add 3 drops of Sudan III stain to each test tube. Shake gently to mix. A red- stained oil layer will separate out and float on the water surface if fat is present.
2. Spot test: Take a small strip of filter paper. Press a small quantity of extracts between the filter paper. Oil stains on paper indicates the presence of fixed oils.
3. Saponification test: To 1 ml of the extract add few drops of 0.5 N alcoholic potassium hydroxide along with a drop of phenolphthalein. Heat the mixture on a water bath for 1-2 hours. The formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

Phenol Test

1. Ferric chloride Test: To 1 ml of the extract, add ferric chloride solution, formation of a dark blue or greenish black color product shows the presence of tannins.

Diterpenes Test

1. Copper acetate test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Test for Saponins

1. Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder

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for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

2. Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

2.3 In-Vitro Anti-Oxidant Activity

Determination of DPPH Scavenging Assay

DPPH radical scavenging activity of Nigella Sativa was determine according to the method reported by Blois [19]. An aliquot of 0.5 ml of sample solution in methanol was mixed with 2.5 ml of 0.5 mm methanolic solution of DPPH. The mixture was shaken vigorously and incubated for 37 min in the dark at room temperature. The absorbance was measured at 517 nm using UV spectrophotometer. Ascorbic acid was used as a positive control. DPPH free radical scavenging ability (%) was calculated by using the formula.

% of inhibition = absorbance of control – absorbance of sample / absorbance of control ×100

Determination of Fecl3 scavenging Antioxidant assay (FSAA)

The ferric chloride scavenging assay was performed according to Benzie and Strain (20) with some modifications. The stock solutions included 300 mm acetate buffer (3.1 g CH3COONa.3H2O and 16 ml CH3COOH), pH 3.6, 10 mM TPTZ (2, 4, 6-Tripyridyl-striazine) solution in 40 mm HCl, and 20 mM FeCl3.6H2O solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution and 2.5 ml FeCl3.6H2O solution and then warmed at 37°C before using. The solutions of plant samples and trolox were formed in methanol (250 mg/mL). 10 mL of each of sample solution and BHT solution were taken in separate test tubes and 2990 mL of FSAA solution was added in each to make total volume up to 3 mL. The plant samples were allowed to react with FSAA solution in the dark for 30 min. Readings of the coloured product [ferrous tripyridyltriazine complex] were then taken at 595 nm. The FRAA values were determined as micromoles of trolox equivalents per mL of sample by computing with standard calibration curve constructed for different concentrations of trolox. Results were expressed in TE µg/mL.

Determination of phosphor- molybdenum scavenging assay

The antioxidant activity of the nigella sativa seed extract was determined by the phosphormolybdenum ,. 0.3 ml of extract was mixed with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mm sodium phosphate and 4 mMammonium molybdate). The reaction mixture was incubated at 95ċ for 90 min and cooled to room temperature. Finally, absorbance was measured at 695 nm using a spectrophotometer (Merck thermo spectronic, Model NO. UV-1, double beam) against blank. Methanol (0.3 ml) in place of extract was used as the blank. The total antioxidant capacity was expressed as the number of equivalents of Ascorbic acid (AAE).

III. RESULTS AND DISCIUSSION

Table 1: Preliminary Phytochemical Evaluation of Lawsenia Inermis and juglance regia leaves Extract

S. No	Phyto-constituents	IdentificationTest	*Juglance regia*	*Lawsonia inermis*
1	Alkaloids	• Mayer test • Wagnertest	-ve -ve	++ve +ve
2	Glycosides	• Legal test • Libbermanbuchard test • salkowskitest • keller killani test	-ve +ve -ve -ve	++ve -ve +ve +ve
3	Tannins	• Vanillin- HCL test • Gelatin test	+ve +ve	+ve -ve
4	Resins	• Turbiditytest • Ferric-Cl test	-ve +ve	-ve -ve

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5	Flavanoids	• Shinodatest • Lead acetatetest • Alkalinetest	+ve -ve +ve	+ve -ve ++ve
6	Steroids	• Salkowskitest • Libermann - reaction	+ve -ve	-ve +ve
7	Amino-acids	• Ninhydrintest • Cysteine test	-ve -ve	-ve -ve
8	Proteins	• Precipitatetest • Biuret Test	+ve +ve	+ve +ve
9	Carbohydrate	• Molish test • Benedicttest	+ve +ve	+ve +++ve
10	Fats & Oil	• Sudan red • spot test • saponificati on test	+ve -ve +ve	+ve ++ve +ve
11	Phenol test	• ferric chloride test	_+ve	++ve
12	Diterpens	• cooper acetate test	-ve	+ve
13	saponins test	• forth test • foam test	-ve +ve	++ve -ve

IV. INVITRO ANTIOXIDANT ACTIVITY

4.1 DPPH Inhibition (%)

Table 2: DPPH Inhibition (%) for Lawsenia inermis extract and Juglans regia extract

Sample	Concentration (µg/mL)	DPPH Inhibition (%)	IC50 (µg/mL)
Lawsenia inermis	25	80.3	12.3
	50	90.7	
	100	95.2	
Juglans regia	25	56.8	65.2
	50	72.6	
	100	85.4	

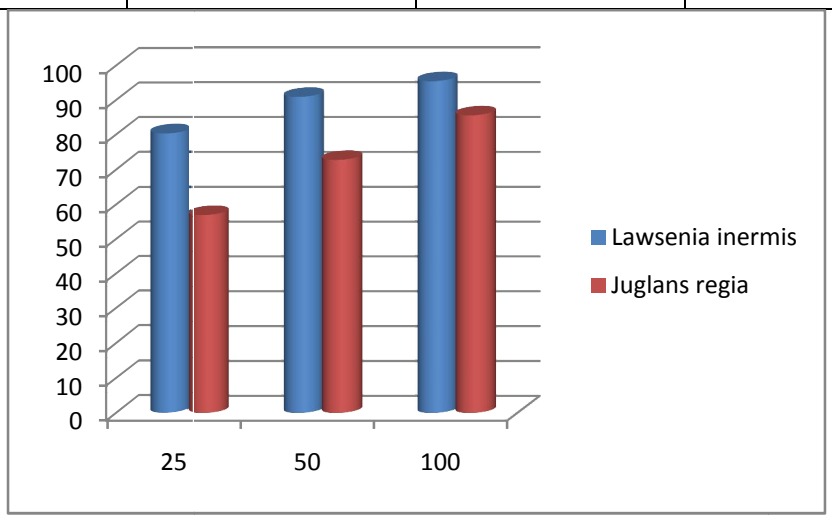

Fig 1: DPPH Inhibition (%) for Lawsenia inermis extract and Juglans regia extract

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Lawsenia inermis extract shows a higher DPPH inhibition percentage than Juglans regia extract, indicating a stronger antioxidant activity. Lawsenia inermis extract also has a lower IC50 value, which means that a lower concentration of the extract is required to scavenge 50% of the DPPH radicals.

4.2 The FeCl3 Radical Scavenging Assay

The FeCl3 radical scavenging assay is used to evaluate the antioxidant activity of a substance by measuring its ability to chelate iron ions and prevent the formation of free radicals.

Table 3: FeCl3 radical scavenging assay for Lawsenia inermis extract and Juglans regia extract

Sample	Concentration (µg/mL)	FeCl3 Inhibition (%)	IC50 (µg/mL)
Lawsenia inermis	25	78.2	13.5
	50	88.9	
	100	94.6	
Juglans regia	25	**	72.3
	50	65.8	
	100	82.1	

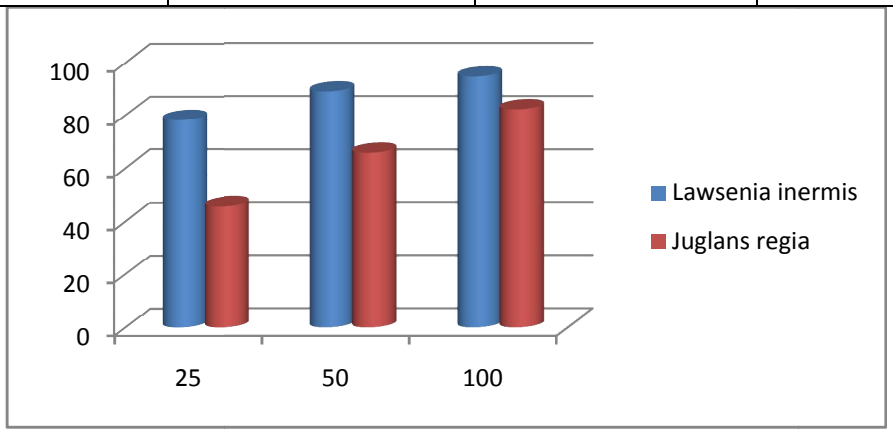

Fig 2: FeCl3 radical scavenging assay for Lawsenia inermis extract and Juglans regia extract

Lawsenia inermis extract shows a higher FeCl3 inhibition percentage than Juglans regia extract, indicating a stronger antioxidant activity. Lawsenia inermis extract also has a lower IC50 value, which means that a lower concentration of the extract is required to scavenge 50% of the FeCl3 radicals.

4.3 The Phosphomolybdenum Assay

The phosphomolybdenum assay is used to measure the total antioxidant capacity of a substance by evaluating its ability to reduce molybdenum (VI) to molybdenum (V).

Table 4: Phosphomolybdenum assay for Lawsenia inermis extract and Juglans regia extract

Sample	Concentration (µg/mL)	Phosphomolybdenum Scavenging (%)	IC50 (µg/mL)
Lawsenia inermis	25	86.2	14.7
	50	92.8	
	100	97.3	
Juglans regia	25	63.7	56.9
	50	75.2	
	100	86.4	

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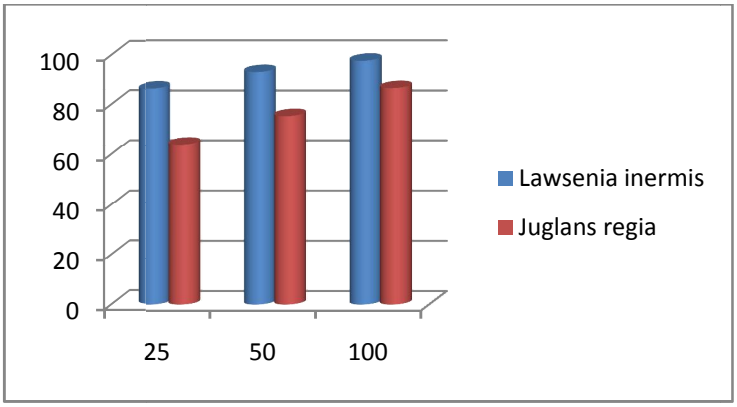

Fig 3: FeCl3 radical scavenging assay for Lawsenia inermis extract and Juglans regia extract

Lawsenia inermis extract shows a higher phosphomolybdenum scavenging percentage than Juglans regia extract, indicating a stronger antioxidant activity. Lawsenia inermis extract also has a lower IC50 value, which means that a lower concentration of the extract is required to scavenge 50% of the phosphomolybdenum radicals.

V. CONCLUSION

In conclusion, this study found that Lawsonia inermis and Juglans regia leaf extracts contain several bioactive compounds that contribute to their antimicrobial and antioxidant activities. The Lawsenia inermis extract demonstrated higher antioxidant activity compared to Juglans regia extract, indicating its potential as a natural source of antioxidants. Future research could focus on identifying the specific compounds responsible for the observed activities, investigating potential synergistic effects, and conducting in vivo studies to validate the extracts' safety and efficacy as therapeutic agents. Furthermore, this study highlights the importance of plants as a source of natural compounds with potential applications in various industries.

Continued research on the phytochemical composition and bioactivities of plants could lead to the development of novel and effective natural products with potential applications in pharmaceuticals, food, and cosmetics. Ultimately, this could contribute to the development of more sustainable and eco-friendly products while reducing reliance on synthetic chemicals and their potential environmental impacts.

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