PHARMACOGNOSTIC AND PHYTOCHEMICAL EXTRACTION OF LAWSONIA INERMIS AND JUGLACE REGIA

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ABSTRACT:

Aim: The present work aimed to investigate the chemical composition of Lawsonia inermis and the Juglans regia. **Methods:** The Powder lawsonia inermis and Juglans regia were evaluated for various physicochemical Constants such as Moisture Content,Total Ash Value, Acid-insoluble ash , Water soluble ash, Alcohol-Soluble Extractive Value and Water-Soluble Extractive Value. The extraction of lawsonia inermis and juglans regia were evaluated for test of alkaloids,tests of glycosides, tests for carbohydrate, tests for tannins,tests for flavonoids, tests for resins, test for steroids, test for proteins and amino-acids, test for fats, phenol test, diterpenes test, and test for saponins. **Results:** The average moisture content of the powdered plant material using loss on drying method was found to be 8.34% and 10.62 %, Ash values obtained include total ash as 7.80% and 5.24% , Acid insoluble ash 6.15 and 3.31%, Water soluble 4.34% and 8.44% . The alcohol and water extractive values were 18.30% and 21.40% of *Lawsonia inermis* and *Juglance regia.* The phytochemical screening of Lawsonia inermis and Juglance regia extracts recorded the presence of compounds carbohydrates, glycosides, triterpenes, flavonoids, saponins and alkaloids present in all extracts. **Conclusion:** These chemical groups are known for their antimicrobial, antifungal, anti-parasitic activities could be responsible for the observed antifungal activities.

KEYWORDS: Lawsonia inermis, Juglans regia, Phytoconstituents, Pharmacognocy, Extraction

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INTRODUCTION:

Pharmacognosy is the systematic study of properties that is structural, chemical and biological effects of crude drugs are studied, method of cultivation, drying, collection, preservation and preparation. Mostly the crude drugs are obtained from different parts of plant or from a whole plant [1].Therapeutic agents are isolated

from different medicinal plant by different way that is modern, Unani, Chinese and traditional system of medicine. On the basis of chemical analysis, modern systems of medicine have negative reaction and traditional system of medicine have no side effect and produce good actions [2] .

Medicinal plants comprise some bioactive organic complexes that is carbohydrates, tannins, flavonoids, alkaloids, steroids and terpenoids which offer precise biological action on the human body. The chemical constituents of plant are helpful because such information was important for the manufacture of complex chemical constituents [3]. Different parts of the plants such as flowers, fruits, leaves, seeds, roots, endocarp and bark are bases for the origin of secondary metabolites. The plants that contains phytochemicals have constantly played a vital role in medicine []. Medicinal plants are used by hakims as 80% of the population living in the villages that are totally dependent on the traditional system of medicines

In the past decade, interest on the topic of antimicrobial plant extract has been growing, and the use of herbal medicines in world represents a long history of human interaction with the environment. The plant used for traditional medicine contains a wide range of substance that can be used to treat chronic as well as infectious disease.[4]

Lawsonia inermis Linn (Henna), the common names in different languages are: Henna (English), lalle (Hausa), lali (Yoruba), mehndi/heena (Urdu), mehndi (Hindi). It is a tropical and subtropical shrub, growing in North Africa. Henna leaves are very popular natural dye to color hand, finger, nails and hairs. The dye molecule, Lawson is the chief constituents of the plant; its highest concentration is detected in the petioles. In folk medicines, henna has been used as astringent, antihemorrhagic, intestinal antineoplastic, cardio-inhibitory, hypotensive, sedative and also as therapeutic against amoebiasis, headache, jaundice and leprosy. Modern pharmacological research on henna and its constituents has confirmed its anti-inflammatory, antipyretic, and analgesic effects. [5]

Juglans regia L. commonly known as “walnut plant” is the wide spread nut tree in the biosphere belongs to family Juglandaceae. It is a distinctive plant having medicinal uses. All parts of walnut can be useful for humans. Previously the Ancient, Greeks and Romans describe and studied the walnut and Theophrastus, “the father of botany” was one of the first to designate walnut [6].

The production of walnut is increase worldwide. The pairs of walnut have been rotated and played with in the palm of the hand to stimulate the circulation blood and as a status symbol in Chinese culture [7].

J. regia L. are used for the preparation of Batch flower remedies, a kind of alternative medicine promoted for its effect on health [8]. Juglone is a chemical release from J. regia L. has toxic properties and it is toxic to different plant species at diverse levels. Juglone is occur in significant quantities in all parts of walnut and in endocarp is very little or absent [9]

MATERIALS AND METHODS:

Plant material and Chemicals

Leaves of Lawsonia inermis and Juglance regia were purchased locally 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 no. 12721 has been deposited in the same. All other ingredients were used analytical grade.

Extraction

Leaves of Lawsonia inermis and Juglance regia 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 extract was evaporated using rotary evaporator. [10]

Determination of Physicochemical Constants of the Powdered Leaves of Lawsonia inermis and Juglance regia

Moisture Content

This is the quantity of moisture present in a plant material. Moisture content of the powdered sample will be determined by loss on drying method. 3.0g each of the powdered sample was accurately weighed and placed in some clean, dried evaporating dishes of known weights. They were placed in an oven and heated at a temperature of 105ºC for 1 hour, then cooled in a dessicator and re-weighed. Heating and weighing were repeated until a constant weight was obtained.[11]

Total Ash Value

2g of powdered plant materials was accurately weighed and placed separately in a crucible of known weight. It was heated gently and the heat gradually increased until it is white indicating the absence of carbon. It was allowed to cool in a desiccator and weighed; this was repeated until a constant weight was obtained.[11]

Acid-insoluble ash

This is the residue that remains after boiling the total ash with dilute hydrochloric acid. This was determined for the powdered plant material. 25ml of dilute hydrochloric acid was added to the crucible containing ash. It was covered with a watch glass and gently boiled for 5mins. The watch glass was rinsed with 5ml of hot water and the liquid added to the crucible. The insoluble matter was collected on an ash less filter-paper and washed with hot water until the filtrate is neutral. The

filter-paper containing the insoluble matter was transferred to the original crucible, dried in an oven and ignited to a constant weight.[11]

Water soluble ash

To the crucible containing the total ash, 25ml of water was added and boiled for 5 minutes. The insoluble matter was collected in a sintered glass crucible. It was then be washed with hot water and ignited in a crucible for 15 minutes at 105oC. The weight of the residue was subtracted from the weight of the total ash. The content of water soluble ash per air dried powdered sample was calculated and recorded (WHO, 2011).[12]

Alcohol-Soluble Extractive Value

This is the amount of extraction in percentage of a plant sample with alcohol. 4g of each of the plant material was separately weighed in a conical flask. 100ml of ethanol was added and macerated for 24 hours, during which the mixture was frequently shaken within the first 6hours using a mechanical shaker. It was filtered and 25ml of the filtrate transferred into an evaporating dish of known weight and evaporated to dryness on a water bath. It was dried to a constant weight, the percentage of alcohol-soluble extractive value was then determined for the plant.[12]

Water-Soluble Extractive Value

This is the amount of extraction in percentage of a plant sample with water. Same procedure as in alcohol-soluble extractive value was repeated here for the two plants, but solvent for extraction here was water.[12]

Elemental analysis of the Powdered Leaves

The elemental analyses of the plant materials were carried out in Ahmadu Bello University Zaria, Multi-user Research Laboratory. Powdered plant material was digested using 2.5ml of hydrochloric acid (HCl) and 7.5ml Nitric Acid (HNO3). The concentration of Fe, Mg, Zn, and Cu was read using the flame atomic absorption spectrophotometer (FAAS), AA 500 model, Atomic Emission Spectrophotometer. Atomic Absorption Spectrophotometer was used for other elements. Before determining the concentration of any element in the sample, calibration curve of the element in the sample was prepared using prepared standard stock solutions for the elements as reported by AOAC.[13]

Preliminary Phytochemical Evaluation of *Lawsonia Inermis* and *Juglance regia* Extract [13]

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 brown precipitate.

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% sodium hydroxide 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 drop of 20% sodium hydroxide solution. Deep red 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 added along 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.

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.

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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 H2SO4 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. H2SO4. 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 of red/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 solution in 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

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 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.

RESULTS AND DISCIUSSION:

Table 2. Physicochemical Constituents of Powdered Leaves of *Lawsonia Inermis and Juglance regia*

Parameter	**Values (% w/w) ± SEM***		**B.H.P STANDARD**
	Lawsonia inermis	***Juglance regia***	

Moisture content	8.34	10.62	<12%
Ash content	7.80	5.24	< 20%
Acid in soluble content	6.15	3.31	
Water soluble content	4.34	8.44	--
Water extractive value	18.30	12.40	--
Ethanol extractive	22.43	19.32	--

Phytochemical investigation

Table 1 - The phytochemical investigation for various chemical constituents in Lawsonia Inermis and Juglance regia extract is given below.

S. No	Phyto-constituents	IdentificationTest	*Juglance regia*	*Lawsonia inermis*
1	Alkaloids	a. Mayer test b. Wagnertest	-ve -ve	++ve +ve
2	Glycosides	a. Legal test b. Libberman buchard test c. salkowskitest d. keller killani test	-ve +ve -ve -ve	++ve -ve +ve +ve
3	Tannins	a. Vanillin-HCL test b. Gelatin test	+ve +ve	+ve -ve
4	Resins	a. Turbiditytest b. Ferric- Cl test	-ve +ve	-ve -ve
5	Flavanoids	a. Shinodatest b. Lead acetate test c. Alkalinetest	+ve -ve +ve	+ve -ve ++ve

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

DISCUSSION:

The physicochemical constants of *Lawsonia inermis* and *Juglance regia* determined include the moisture content, total ash value, acid insoluble ash, water soluble ash, alcohol (ethanol) extractives value and water extractives value. These values are useful as criteria to evaluate the identity and purity of crude drugs. The average moisture content of the powdered plant material using loss on drying method was found to be 8.34% and 10.62 % of *Lawsonia inermis* and *Juglance regia respectively* , and this value is within the permissible limits because B. H. P, (1990) and WHO, (2011) recommend the percentage of moisture content in any crude drug to be within 12-14 %. Low or permissible moisture in crude drugs may discourage the growth of bacteria, yeast, mould and fungi and will stand for long period of time during storage without spoilage or suggesting better stability against degradation of product (WHO, 1996).

Ash values obtained include total ash as 7.80% and 5.24%, of *Lawsonia inermis*

and *Juglance regia.* Acid insoluble ash 6.15 and 3.31% of *Lawsonia inermis* and *Juglance regia.* Water soluble 4.34% and 8.44% of *Lawsonia inermis* and *Juglance regia.* These Ash values indicate the presence of various impurities such as carbonate, oxalate, sand and silicate in plant materials. The non-physiological ash is the inorganic residues in water soluble ash after the plant drug is burnt while the acid insoluble ash indicated that the plant was in good physiological condition and it contained little extraneous matters compared to the total ash content. The total ash value is used as a standard to assess the identity and purity of crude drugs (WHO, 1996, WHO, 2011). The alcohol and water extractive values were 18.30% and 21.40% *Lawsonia inermis* and *Juglance regia* respectively. It was observed that alcohol had a higher extractive value (21.40%).

The phytochemical screening of Lawsonia inermis and Juglance regia extracts recorded the presence of compounds carbohydrates, glycosides, triterpenes, flavonoids, saponins and alkaloids present in all extracts. and responsible for antimicrobial ,anti-parasitic activities as reported in similar studies.

CONCLUSION:

The present study provides the the physicochemical,phytochemical and pharmacognostic evaluation of lawsonia inermis and juglance regia. All parameters are withins limits and as per guidelines of concern authorities.All phytochemicals presents in lawsonia inermis and juglance regia can be used as their exhibit pharmacological activities. Further studies are require to explore the beneficial uses of lawsonia inermis and juglance regia.

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Conflict of Interest

None.

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