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Qualitative phytochemical studies of the Libyan henna leaves (*Lawsonis inermis* Linn.)

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Abstract The plant *Lawsonia inermis Linn.* (Henna) belongs to the family Lythraceae. *L. inermis* (LI) is a glabrous splited plant or small tree, has been traditionally well used established aesthetic agent frequently used in colouring hair, skin and nails and also exhibits antimicrobial & anti-dermatological properties. The plant is widely distributed throughout the tropical and subtropical places of North Africa, Middle East and Indian subcontitent. The plant contains carbohydrates, phenolic, flavanoids, saponins, proteins, alkaloids, terpenoids, quinones, coumarins, xanthones, fat, resin and tannins are the main phytoconstituents. A scrutiny of literature revealed some notable pharmacological activities like antibacterial, antifungal, antiparasitic, antioxidant, hepatoprotective, central nervous, analgesic, anti-inflammatory, antipyretic, wound and burn healing, antiurolithiatic, antidiabetic, hypolipidemic, antiulcer, antidiarrhoeal, diuretic and anticancer activities. This study deals with the preliminary qualitative phytochemical screening of leaf of *L. inermis* (LI) which includes TLC, UV and FTIR fingerprinting. Qualitative phytochemical analysis revealed the presence of alkaloids, steroids, flavonoids, triterpenoids, tannins and saponins. The total phenolic, flavonoid, proanthocyanidin, alkaloidal, and saponin contents of methanolic extract of *L. inermis* were measured in this study. The UV-VIS profile showed different peaks ranging from 200-800nm with different absorption respectively. The FTIR spectrum confirmed the presence of alcohols, phenols, alkanes, alkenes, carbonyl, carboxylic acids and aromatic compounds in extract. These studies will provide referential information for the correct identification of the crude drugs.

Keywords *Lawsonia inermis Linn*; phytochemical screening; TLC, UV, FTIR

Introduction

The importance of medicinal plant in drug development is known to us and humans have used them for different diseases from the beginning of human history [1]. Traditional folk treatment from wild plants has always guided researchers to search for novel medications to develop healthy life for humans and animals. The knowledge gathered by generations was either documented or passed on to the posterity and this practice was generally termed as "Traditional Medicine" [2]. The World Health Organization (WHO) estimates that about 80% of the population living in the developing countries relies almost exclusively on traditional medicine for their primary health care needs. In almost all the traditional medicines, the medicinal plants play a major role and constitute the backbone of the traditional medicines [3]. The modern (Allopathic) system of medicine is based on drastic cures through synthetic drugs and chemical compounds. It is noteworthy that since last twenty-five years or so, a notable drop in the popularity of this system is noticeable. This trend can be attributed to the increasingly harmful side effects induced by some of these synthetic drugs. There is a famous line that says, "One man's

Aspirin is another man's peptic ulcer" [4 -7]. It is now known that chronic ailments, which require long-term treatments, are not always cured by allopathic drugs. Moreover, the synthetic drugs and intermediatery chemicals are extremely expensive [8]. For these and other reasons synthetic drugs are being widely replaced with the medicinal plants which are non-polluting renewable resources and are the only hope for sustainable supplies of cheaper medicines for the world's growing population. Plants are also appreciated in pharmaceutical research as the major resource for new medicine and a growing body of medical literature supports the clinical efficacy of herbal treatments [9]. Today, about 40% doctors, especially in India and in China (the Mystic Orient) have reverted to increasing use of indigenous drugs and natural medicines. Steadily, a sizeable section of scientists in biological, biochemical and biomedicinal discipline have embarked on research on medicinal plants, which are the staple sources of many indigenous drugs [10].

Lawsonia inermis Linn (Family: Lythraceae) which is commonly known as henna, mainly present in subtropical and tropical areas and is used in all over the world. It was used for over 9000 y for its cosmetic values as a dye [11]. *Lawsonia inermis* is generally considered as a native of Africa and Asia. It was distributed in Africa: Egypt, Ethiopia, Somalia, Sudan, Zaire,Niger, Benin, Burkina Faso, Cote D'Ivoire, Gambia, Ghana, Guinea, Guinea-Bissau, Liberia, Mali, Nigeria, Senegal, Sierra Leone, Togo, South Africa, Comoros, Seychelles; Asia: India, Pakistan, Sri Lanka. It is widely cultivated in tropical regions of the world, North and East Africa, the Arabian Peninsula, the Southern areas of the Middle East, and South Asia [12]. The phytochemical analysis of Lawsonia inermis revealed the presence of carbohydrates, phenolic, flavanoids, saponins, proteins, alkaloids, terpenoids, quinones, coumarins, xanthones, fat, resin and tannins. It also contained 2-hydroxy-1,4-naphthoquinone (lawsone). Many alkaloids, napthoquinone derivatives, phenolics and flavonoids were isolated from different parts of *Lawsonia inermis* [13]. The pharmacological studies showed that *Lawsonia inermis* showed antibacterial, antifungal, antiparasitic, molluscicidal, antioxidant, hepatoprotective, central nervous, analgesic, anti-inflammatory, antipyretic, wound and burn healing, immunomodulatory, antiurolithiatic, antidiabetic, hypolipidemic, antiulcer, antidiarrhoeal, diuretic, anticancer and many other pharmacological effects. [14] Hence, the present study was designed to study the qualitative phytochemical screening TLC, UV and FTIR profile of extracts.

Materials and Methods

Description of *Lawsonia inermis* Linn

This plant is multi branched, deciduous shrub or small tree having 2.6 m height. Leaves of this plant are 1.3-3.2cm broadly or elliptic lanceolate. Flowers are white or rose colored, which are used as a fragrant agent in local scent. Pedicle is short less than 1.3 cm, numerous in number and slender in shape. Calyx is 3-5 mm, long broadly campanulate; lobes are 2.5-3 mm, long, suborbicular or subreniform and undulate. Stamens are 8, which are inserted in pairs on the calyx-tube. Capsules are slightly veined
outside, globose and diameter of the capsule is 6 mm. Persistent calyx support capsule with the tipped style. Pea shape and globose seed capsules figure 1(A & B) [15].

Figure 1: Exomorphic feature of L. inermis

Collection and Authentication of Plant Material

For research and experiment, we collect fresh leaves of plant *L. inermis* from the basin city of the Mediterranean sea, Misurata, Libya in March 2019, which was authenticated by Dr. Huda Elgubbi, Department of Botany, College of Science, Misurata University, Misurata, Libya and for the records a voucher specimen No. HC 59/01 has been submitted in the herbarium, Department of Botany, College of Science, Misurata University, Misurata, Libya

Extraction and Fractionation

The leaves of plant *L. inermis* (0.5 kg) was shade dried, coarsely powdered and extracted exhaustively with 95% methanol in a Soxhlet apparatus. The methanolic extract of the plant was then concentrated on a steam bath and dried under reduced pressure to get 5.9 g of dark brown mass.

Phytochemical Qualitative Analysis

The plant extracts and methanolic and ethanolic aqueous solutions were assessed for the existence of the phytochemical analysis by using the following standard methods [16-19].

Test for Anthraquinones

10 ml of benzene was added in 6 g of the Ephedra powder sample in a conical flask and soaked for 10 minutes and then filtered. Further 10ml of 10% ammonia solution was added to the filtrate and shaken vigorously for 30 seconds and pink, violet, or red color indicated the presence of anthraquinones in the ammonia phase.

Test for Tannins

10 ml of bromine water was added to the 0.5 g aqueous extract. Decoloration of bromine water showed the presence of tannins.

Test for Saponins

5.0 ml of distilled water was mixed with aqueous crude plant extract in a test tube and it was mixed vigorously. The frothing was mixed with few drops of olive oil and mixed vigorously and the foam appearance showed the presence of saponins.

Tests for Flavonoids

Shinoda Test- Pieces of magnesium ribbon and HCL concentrated were mixed with aqueous crude plant extarct after few minutes and pink color showed the presence of flavonoid.

Alkaline Reagent Test- 2 ml of 2.0% NaOH mixture was mixed with aqueous plant crude extract; concentrated yellow color was produced, which became colorless when we added 2 drops of diluted acid to mixture. This result showed the presence of flavonoids.

Tests for Glycosides

Liebermann's Test- We added 2.0 ml of acetic acid and 2 ml of chloroform with whole aqueous plant crude extract. The mixture was then cooled and we added H2SO4 concentrated. Green color showed the entity of aglycone, steroidal part of glycosides.

Keller-Kiliani Test- A solution of glacial acetic acid (4.0 ml) with 1 drop of 2.0% FeCl3 mixture was mixed with the 10ml aqueous plant extract and 1mlH2SO4 concentrated. A brown ring formed between the layers which showed the entity of cardiac steroidal glycosides.

Salkowski's Test- We added 2 ml H_2SO_4 concentrated to the whole aqueous plant crude extract. A reddish brown color formed which indicated the presence of steroidal aglycone part of the glycoside.

Test for Terpenoids

2.0 ml of chloroform was added with the 5 ml aqueous plant extract and evaporated on the water path and then boiled with 3 ml of H_2SO_4 concentrated. A grey color formed which showed the entity of terpenoids.

Test for Steroids

2 ml of chloroform and concentrated H_2SO_4 were added with the 5 ml aqueous plant crude extract. In the lower chloroform layer red color appeared that indicated the presence of steroids.

Test for Carbohydrates

a) Molisch test

Small quantities of alcoholic and aqueous extracts were dissolved separately in 5 ml of distilled water and filtered. To this solution 2-3 drops of α-naphthol solutions were added followed by about 1ml of concentrated sulphuric acid along the sides of inclined test tube so as to form two layers and observed for formation of violet colored ring at the interface.

b) Fehling reagent (Detection of reducing sugar)

Few drops of Fehling solution (alkaline solution of cupric ion complexed with tartrate ion) were added in dilute extracts and heated for 30 minutes and observed for formation of brink red colored precipitate.

Test for phenolic compounds

Ferric chloride solution

The extracts were taken in water and warmed; to this 2ml of ferric chloride solution was added and observed for the formation of green and blue color.

Lead acetate solution

To the extract (2 ml) lead acetate solution was added and observed for the formation of precipitate.

Gelatin solution

A few ml of Gelatin Solution was added to the aqueous extract and observed for the formation of precipitate or turbidity.

Thin layer chromatography (TLC)

Thin layer chromatography studies of the ethanol and chloroform extracts carried out in various solvents at 30^{O} C using Silica gel G as adsorbent and the R_f values were determined [20].

Determination of total phenolic contents

The total phenolic content was determined according to the method described by Singleton (1999) [21]. A suitable aliquot of the methanolic extract was placed in test tubes and made up to 1 ml with distilled water. Then, 0.5 ml Folin-Ciocalteu reagent (1:1 with water) and 2.5 ml sodium carbonate solution (20%) were added sequentially to each tube. Then, the tubes were vortexed for 2 min, kept in the dark for 40 min and the absorbance was recorded at 725 nm. The amount of total phenolics was calculated as gallic acid equivalents /mg of extract.

Determination of total flavonoid contents

Flavonoid contents were measured using a modified colorimetric method of Chang *et al*., [22] Extract solution (0.25 ml, 1 mg/ml) was added to a test tube containing 1.25 ml of distilled water. Sodium nitrite solution (5%, 0.075 ml) was added to the mixture and maintained for 5 min. Then, 0.15 ml of 10% aluminum chloride was added. After 6 min, 0.5 ml of 1 M sodium hydroxide was added. The mixture was diluted with 0.275 ml of distilled water. The absorbance of the mixture at 510 nm was measured immediately in comparison to a standard curve prepared using quercetin. The flavonoid contents were expressed as mg quercetin equivalent (QE)/g dry basis.

Determination of proanthocyanidin contents

Proanthocyanidin content was determined by the procedure of Sun *et al*.[23] Five hundred microliters of methanolic extract solution was mixed with 3 ml of 4% vanillin–methanol solution and 1.5 ml hydrochloric acid. The mixture was allowed to stand for 15 min. The absorbance was measured at 500 nm, while the final result was expressed as mg catechin equivalent (CE)/g dry basis.

Determination of alkaloids contents

Alkaloid content was determined by the procedure of Harborne [24] Five grams of the sample was weighed into a 250-ml beaker, and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle, and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Determination of saponins contents

Saponin content was determined by the procedure of Obadoni and Ochuko [25]. The samples were ground and 20 g of each were put into a conical flask and 100 ml of 20% aqueous ethanol was added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over a water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. Sixty milliliters of methanolic extract of *L. inermis* was added. The combined methanolic extract were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage.

UV-VIS Spectrum analysis

UV-visible spectrophotometric analysis was conducted on the *L. inermis* extract using a UV-visible spectrophotometer (Perkin Elmer, USA Model: Lambda 950) with a slit width of 2nm, using a 10-mm cell at room temperature. The extract was examined under visible and UV light in the wavelength ranging from 300-800nm for proximate analysis. For UV-VIS spectrophotometer analysis, the extract was centrifuged at 3000 rpm for 10 min and filtered through Whatman No. 1 filter paper. The sample is diluted to 1:10 with the same solvent [26].

FTIR Spectroscopic analysis

Fourier transform infrared (FTIR) was used to identify the characteristic functional groups in the extract. It provides the information about the structure of a molecule could frequently be obtained from its absorption spectrum. A small quantity of the Mentha spicata extract was mixed in dry potassium bromide (KBr). The mixture was thoroughly mixed in a mortar and pressed at a pressure of 6 bars within 2 min to form a KBr thin disc. Then the disc was placed in a sample cup of a diffuse reflectance accessory. The IR spectrum was obtained using Bruker, Germany Vertex 70 infrared spectrometer. The sample was scanned from 4000 to 400 cm-1 [27]. The peak values of the FTIR were recorded.

Results and Discussion

Qualitative Phytochemical Analysis

The present study revealed that the methanolic extract of *L. inermis* revealed the presence of alkaloids, glycosides, flavonoids, phenols, steroids, amino acids, tannins, terpenoids, and carbohydrates (Table 1).

Table 1: Qualitative phytochemical Screening of *L.inermis*

Constituents	**Methanolic extract of *L.inermis***
Alkaloids	+
Carbohydrates	+
Glycosides	+
Tannins	+
Flavonoids	+
Terpenoids	+
Saponins	-
Sterols	+

Proteins	_
Resins	+
Lipids/ Fats	-
Mucilage	-

+ = Present, - = Absent

Thin layer chromatography (TLC)

Thin layer chromatography of the methanolic extracts of *L. inermis* was carried out various solvent system and the Rf values were recorded Table 2. The visualizing reagent employed was anisaldehyde-sulphuric acid reagent to effect visualization of the resolved spots.

Table 2: TLC fingerprints of methanolic extracts of *L. inermis*

Solvent system	No. of spot	R_f value
Benzene: Chloroform (10:90)	3	0.4, 0.3, 0.2
Benzene: Chloroform (40:60)	2	0.9, 0.7
Methanol: Chloroform (10:90)	-	-
Ethyl acetate: methanol (80:20)	-	-
Ethyl acetate: methanol (30:70)	2	0.9, 0.7
Chloroform: ether: acetic acid (40:60:10)	2	0.9, 0.7

Determination of total phenolics, flavonoid, proanthocyanidin, alkaloid, and saponin contents

The content of total phenolics, flavonoids, proanthocyanidin, alkaloid, and saponin was determined in the methanolic extract of *L. inermis* by the proposed method and the results obtained are summarized in Table 3.

Table 3: Determination of total phenolics, flavonoid, proanthocyanidin, alkaloid, and saponin contents

Quantitative parameters	Methanolic extract of *L. inermis*
Phenolics (µg/ml)	0.961 ± 1.01
Flavonoids (µg/ml)	61.71 ± 0.31
Proanthocyanidin (µg/ml)	10.53 ± 0.34
Alkaloids (% w/w)	1.46 ± 0.18
Saponin (% w/w)	0.61 ± 0.31

UV-VIS Analysis

The UV-VIS analysis preformed for identification of phytoconstituents present in methanolic extract of *L. inermis*. The UV-visible spectra were performed to identify the compounds containing σ-bonds, π-bonds and lone pair of electrons, chromophores and aromatic rings. The qualitative UV-VIS profile of methanolic extract of *L. inermis* was taken at the wavelength of 200 nm to 800 nm due to the sharpness of the peaks and proper baseline. The profile showed the peaks at 245, 271, 283, 307, 320, 413, and 666 nm with the absorption 1.309, 1.463, 0.1, 0.066, 0.108 and 0.625 respectively. Figure 1 shows the absorption spectrum of *L. inermis* extract and these are almost transparent in the wavelength region of 300-800 nm. Absorption bands observed pertaining to In the UV-VIS spectra the appearance of one or more peaks in the region from 200 to 400 nm is a clear indication of the presence of unsaturated groups and heteroatoms [28]. The spectrum for *L. inermis* extract shows two peaks at positions 413 nm, and 666nm can be attributed to $\pi \rightarrow \pi^*$ transitions; other absorptions at 413 and 666 nm in the vis regime may be due to $n \rightarrow \pi^*$ transitions of carbonyl group in *L. inermis*. This confirms the presence of organic chromophores within the *L. inermis* extract. Nevertheless, the use of UV-visible spectrophotometery in the analysis of complex media is limited by the inherent difficulties in assigning the absorption peaks to any particular constituents in the system. Thus, UV– VIS findings must be supplemented with some other analytical technique such as GC/MS etc, to enable proper extract characterization and

constituent identification[29]. The obtained data of UV-VIS spectroscopic analysis in the methanolic extract of *L. inermis* shown in figure 2.

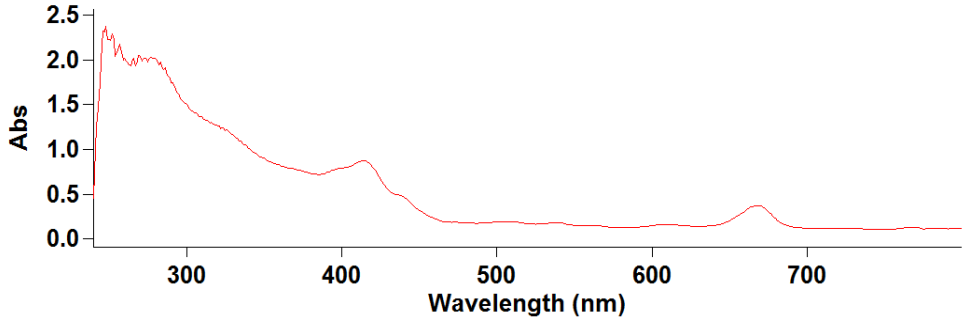

Figure 2: UV-vis absorption spectrum of methanolic extract of L.inermis

FTIR Analysis

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The FTIR spectrum of the *L. inermis* plant extract in the form of KBr pallet is shown in Figure 3. The absorption at 3328.81 cm-1 is due to the stretching of hydroxyl groups that are present in the extract. The band at 2922.23 and 2853 cm-1 is due to the symmetric stretching of saturated (sp3) carbon. The band at 1732 cm-1 is assigned to the carbonyl group of *L. inermis*. The band at 1604 cm-1 is due to C=C stretching associated with the aromatic skeletal mode of the extracts. The vibrational absorption band at 1384.66 cm–1 was assigned to rocking of methyl group. A notable band at 1253 and 1054 cm-1 can be assigned to C-O stretching. A band at 599.76 cm-1 represent the aromatic H out of plane bending [30, 31].

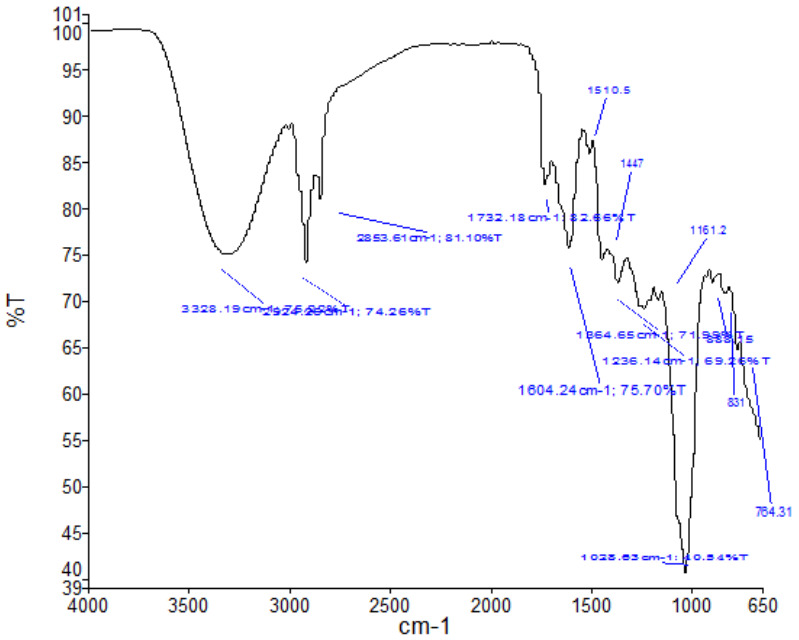

Figure 3: The FTIR spectrum of methanolic extract of L.inermis

Conclusion

This investigation has given preliminary information to determine the chemical composition of *L. inermis* using qualitative UV-VIS, FTIR and techniques. The presence of these bioactive compounds in *L. inermis* plants lends credence to its use by the human community. It also holds for the production of novel drugs with isolation of specific compounds. It could be concluded that *L. inermis* contains various bioactive compounds. The methanolic extract of *L. inermis* sample under FTIR and UV-VIS spectroscopic technique showed that the

presence of phenolic compound and flavonoid which can be isolated and further screened for different kind of biological activities depending their therapeutic uses.

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