Characterization of *Lawsonia inermis* (Henna) as Vegetable Tanning Material

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***Abstract*— Phytochemical studies on the chemistry of polyphenolic constituents, isolation and identification have revealed that henna leaves contain condensed tannin (11.12%). The components leucocyandin, epicatechin, catechin and quercitrin were identical with that of the authentic samples. Main colouring substance of henna (The lawsone) was identified as 2-hydroxy-1,4-naphthoquinone.Analysis of henna leaves extract was studied by ultraviolet (UV). The spray drying of aqueous henna leaves extract gave about 33-35% yield, a brown and fine powder using the co-current spray drier apparatus.**

Index Terms*— *Tannin analysis, Lawsonia inermis, Spray drying

I. INTRODUCTION

The term tannin was first introduced by Seguin to indicate various plants extracts which have the capacity to convert hides and skins into leather [1]. The tannins are widely distributed in nature and occur in different parts of the plant, barks (wattle), roots (canaigre), fruits (myrobalan), leaves (sumac), pods (tara) and cups (velonia)...etc.Tannins occur throughout the greater part of plant Kingdom, and are more prevalent among the higher plants or angiosperm, especially in certain dicotyledonous families [2].

Vegetable tannins are important as retanning agent in the leather production and have been recognized as an important tanning agent in non-chrome tanning. Commercial vegetable tannins are not capable of radically changing the quality of the usual leather products, so that the appearance of a new vegetable tannin is of great important. Characterization of vegetable tannins is important in regard to new tannin for use in leather production. Vegetable tannins were previously classified by means of chemical tests viz, ammonium sulphide solution, limewater, concentrated sulphuric acid, hydrochloric acid-formaldehyde, acetic acid-lead acetate, or bromine water [3]. Coloration using Iron (II) chloride is well known in leather chemistry [4]. It has been reported that the condensed type mainly consist of flavan-3-ol units condensed at 4- and 8-positions. The hydrolysable tannin types were classified into the gallotannins and ellagitannins type [5], [6]. While vegetable tannins are the mixture of complex phenolic compounds, their main components have been gradually identified [5],[6],[7]. Chromatography is the only convenient means of qualitative analysis and the two-way paper chromatography or thin layer chromatography are normally employed to produce "finger print" which is then compared with chromatogram of known materials [8]. Ultraviolet (UV) spectroscopy was used to determine a part of constitution of vegetable tannins [9], [10]. Analysis of various vegetable tannins proceeded by means of ultraviolet, Fourer transform infrared spectroscopies (FTIR) and liquid chromatography (LC). Molecular weights (obtained by means of LC) showed no distinguishing differences amongst various tannins. By using UV spectra it was possible to determine the type of vegetable tannins. The FTIR spectra of each vegetable tannin showed the characteristic absorption patterns, which also allowed us to characterize individual tannins [11]. Also analysis of tan, non-tan, insolubles, moisture content from the various parts of the plant, bark, leaves, wood...etc were carried out by official methods of the analysis [12].

Traditionally the tannins are extracted with water as solvent in open vats at moderate temperature. However, water used for extraction (leaching) should not contain iron and should be soft [13]. The commercial vegetable tannin extracts or "tan liquors" as they are popularly known in the leather industry are mixtures of polyphenolic compounds with a definite T/NT ratio, astringency etc, along with other plant products.

The manufacture of the vegetable tannin extract is essentially based on the extraction of tannins by using a suitable solvent,

usually water, followed by concentration and spray drying to get powder or solidification to get solid (block) extract (**Figure 1**). [13]

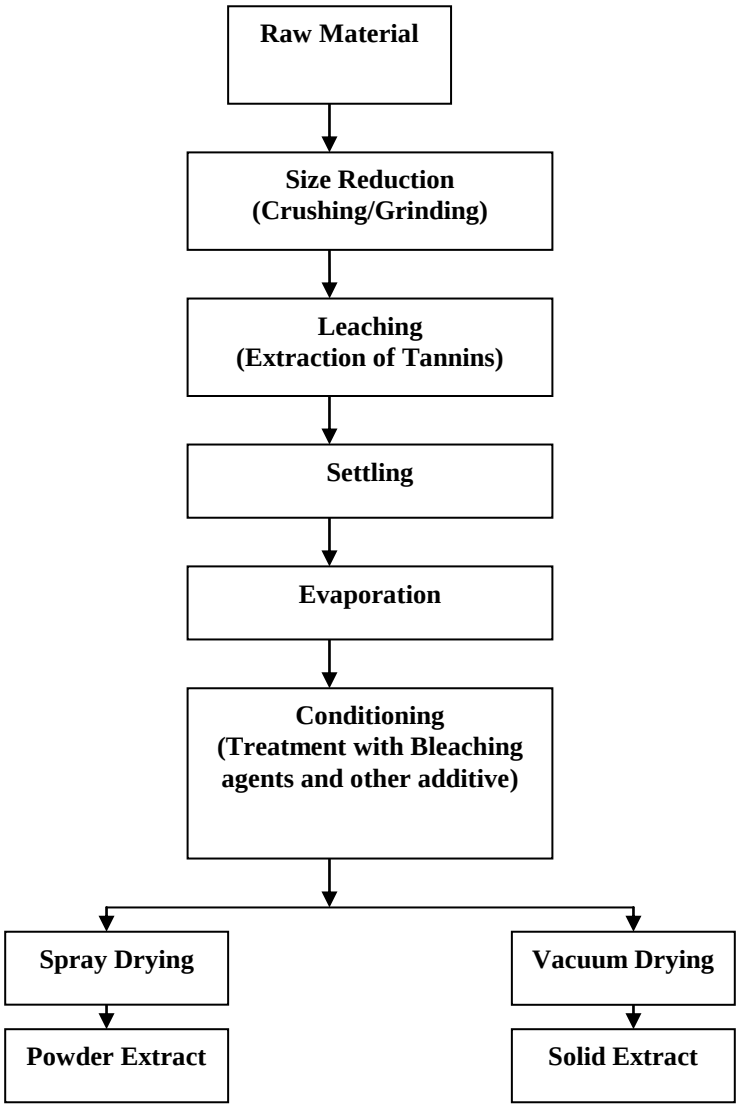

Figure 1: Process Block Diagram for the Manufacture of Vegetable Tannin Extracts.

Lawsonia inermis (Henna) is a member of the family Lythraceae which consists of about 500 species, widely spread in tropical regions with relatively few species in temperate regions [14]. *Lawsonia inermis* is generally considered as a native of Africa and Asia. It is widely cultivated in tropical regions of the world in Sudan, Egypt, China, and India. Major producing countries include Sudan, Egypt and India [15]. Henna plant grows on any type of soil, from light loam to clay loam, but does best on heavy soils, which are retentive of moisture. It tolerates a little alkalinity in the soil. Propagation is carried out through seeds and cuttings [16]. Henna leaves have been extensively used for centuries in the Middle East, the Far East and Northern Africa as dye for nails, hands, hair and textile [17]. Henna is also used in treating skin problems, headache, jaundice, amebiasis and enlargement of the spleen [17]. Leaves of Lawsonia inermis provide an important cosmetic dye. *Lawsonia inermis* has been well investigated phytochemically by various researchers. The occurrence of β-sitosterol glucoside , [18] flavonoids , [17] quinoids , [19] naphthalene derivatives , [20] gallic acid (Figure 2), [19] coumarins [21] , and xanthones [22] in *Lawsonia leaves* has been reported. Earlier work establishes the use of henna as an alternative vegetable retanning agent [23].

In this paper henna leaves widely distributed in Sudan has been studied by different phytochemical methods to show the characterizations of henna leaves.

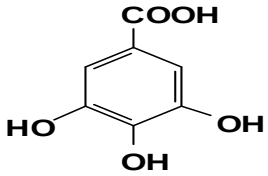

Figure 2: Gallic acid

II. MATERIALS AND METHODS

Lawsonia inermis leaves were collected from Damar area in the Northern Sudan. The air dried leaves were ground and used for determination of ,moisture content, total solid , total soluble, insoluble, tannin, non-tannin, pH, and colour. Chemicals used for the analysis were of analytical reagent. Gelatin test, Iron Alum solution test and Formeldehyde-hydrochloric acid test were carried out to determine the type of tannin of the henna extract solution of analytical strength (0.4%).

For analysis of tannin and non tannins, the gravimetric method, [24] based on the absorption of tannins by hide powder has been used for the study. 2 litres of the henna extract solution were collected and obtained in 4 hours using Procter extractor. Determination of moisture content (%) has been carried out for henna leaves powder [25].

From the 2 liters henna extract solution, unfiltered tannin extract was used for determination of total solids. [26] Also the filtered tannin extract was used for determination of total solubles. [27] The difference between the percentage of total soluble plus percentage moisture and 100 determine the insoluble of henna extract. [28]

The chrome tanned hide powder used for determination of non-tannin.[29] The tannin matter absorbed by the hide powder was determined by the difference between the percentage of total soluble and non tannin [30]. The pH of henna extract solution [31] and the colour [32] were determined using pH meter and Lovibond tintometer respectively.

Two Dimensional paper chromatography was carried out for ethyl acetate fraction of 70% acetone henna leaves extract using solvent systems (A) 6% acetic acid in the first way

followed by (B) n-butanol:acetic acid: water (BAW, 4:1:5 v/v upper layer) in second way. The chromatograms were sprayed with mixture of ferric chloride-potassium ferric, also thin layer chromatography was carried out. Spectroscopic Analysis; Ultra violet absorption spectrum of aqueous henna extract was recorded using Cary 100 Perkin-Elmer UV–visible spectrophotometer; and the Fourier transform Infra Red (FT-IR) instrument, Perkin Elmer spectrum RXI FT-IR was used to establish functional groups in sample of Spray drying henna powder mixed with potassium bromide.

20% aqueous solution of henna leaves extract was prepared, allowed to settle and filtered. The henna extract was spray dried using co-current spray drier apparatus (capacities of spray drier, 500 ml/hr water evaporation) (**Figure 3**).

Figure 3: Co-current spray drier apparatus

III. RESULTS AND DISCUSSION

The iron alum solution test the henna extract gave green color, which is clearly indicative of the condensed tannin presence. Formaldehyde-hydrochloric acid test gave precipitate with henna extract which is clearly indicative of the condensed tannin presence. The percentages of tannin, non-tannin, total soluble, total solids, insoluble, pH, color and the moisture of the henna leaves are shown in **Table 1**.

Preliminary screening proved that ethyl acetate accumulated the bulk of phenolic compounds. *Lawsone* was an amorphous material appeared as an orange spot in the silica gel GTLC (R_f = 0.82 petroluem ether: ethyl acetate) in visible light. This compound was identical with an authentic sample of lawsone. Two dimensional paper chromatography of ethyl acetate fraction of 70% acetone henna leaves extract in **Fig 4** show 1. Leucocyandin R_f 0. 73 in (BAW) and R_f 0.46 in (6% acetic acid) 2. Catechin R_f 0. 72 in (BAW) and R_f 0.42 in (6% acetic acid) 3. Epicatechin R_f 0. 60 in (BAW) and R_f 0.36 in (6% acetic acid) 4. Quercitrin R_f 0.77 in (BAW) and R_f 0.17 in (6% acetic acid) .The R_f of these compounds were found to be identical with that of the authentic samples.

Table 1
Tannin analysis of henna leaves

S.No.	Characteristics	Henna leaves
1.	Tannin, %	11.12
2.	Non-tannin, %	22.64
3.	Total soluble, %	33.76
4.	Total solid, %	36.72
5	Insoluble, %	56.66
6	Moisture, %	9.58
7	pH	4.5
8	Color	Red =1.7
	Lovibond Tentometer	Yellow = 2.8

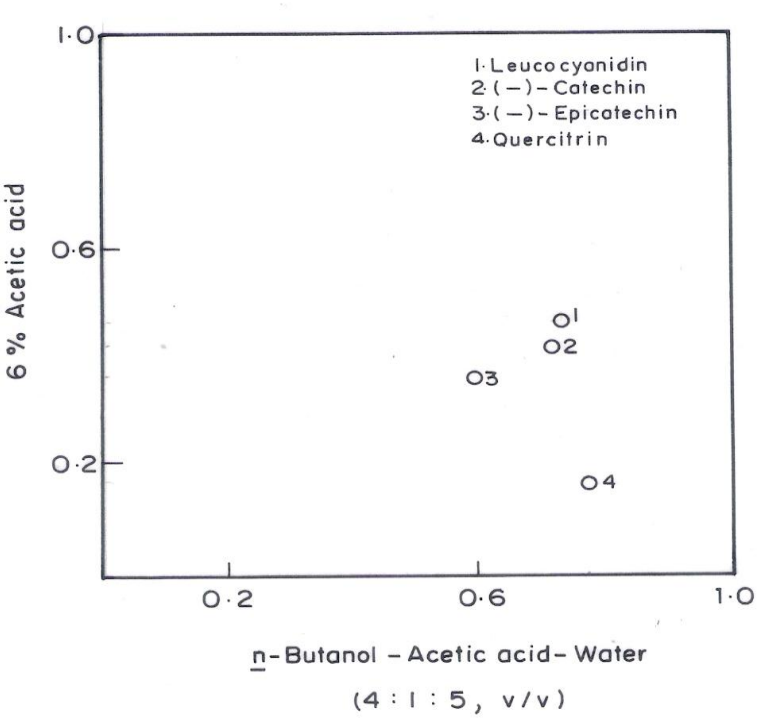

Figure 4: Two Dimensional Chromatogram of ethyl acetate fraction of 70% acetone henna leaves extract.

Ultra violet (uv) spectrum was recorded using Cary 100 Perkin-Elmer UV–visible spectrophotometer. The **Figure 5** showed uv λ_{max} of the aqueous henna extract at 267 nm. The spectral data in the **Fig 6** indicated the presence of hydroxyl groups (ν max 3400 – 2924 cm^{-1}) , absorption at 2363 cm^{-1} (C=C) ,aromatic ring showed the carbonyl(C=O) (ν max 1728-1625cm^{-1}) and absorption at 1420, 1041, 633 cm^{-1}.

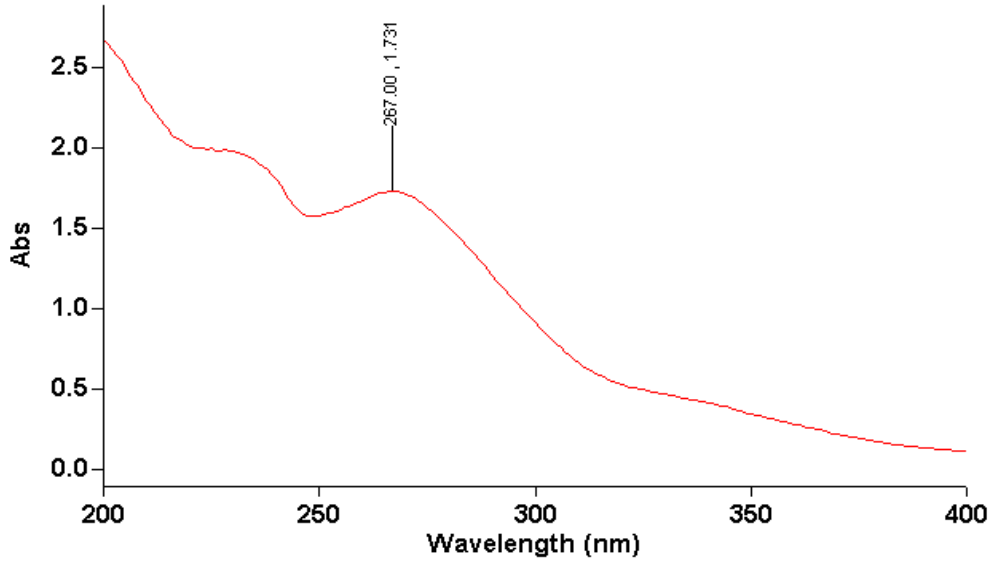

Figure 5: Ultra violet absorption spectrum of aqueous henna extract

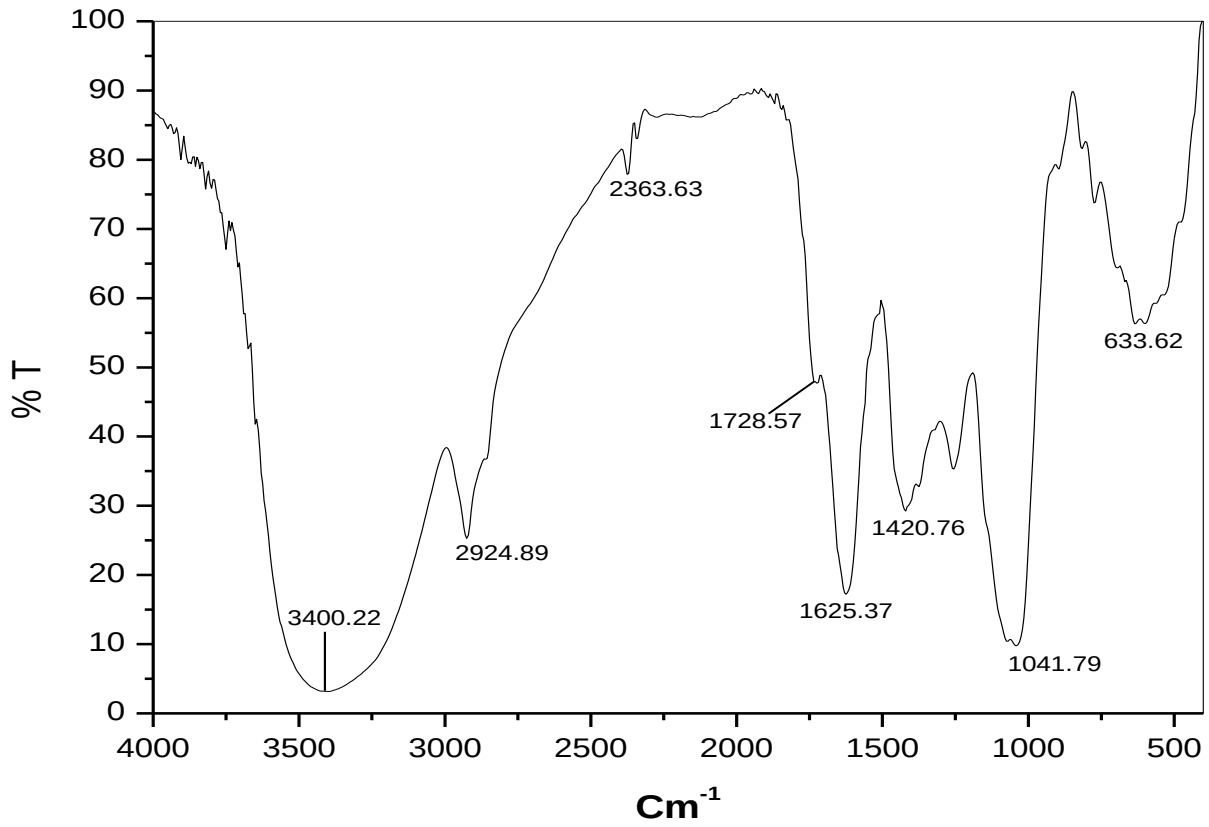

Figure 6: IR spectrum of henna leaves extract spray powder

spray drying of the aqueous henna leaves extract gave about 33-35% yield, a brown and fine powder (**Fig 6**) using the co-current spray drier apparatus. The henna power was hygroscopic and it can absorb moisture if it kept outside. Addition of anhydrous sodium sulphate to the henna extract before spraying can overcome this drawback and gave lightly brown color. Also the addition of sodium sulphite gave lightly brown color. The spray drying powder is packed in dark container for safe preservation. Thus it is possible by this technique to recover a soluble power from aqueous henna leaves extract.

Figure 7: Henna leaves spray powder

IV. CONCLUSION

The analysis of henna leaves powder show that the tannin content, non tannin, TSS, TS, moisture content, and pH was 11.12%, 22.64%, , 33.76%, 36.72%, 9.58 %, 4.5 respectively and color (Lovibond Tentometer), Red =1.7, Yellow = 2.8.The phytochemical investigation of the leaves of *Lawsonia inermis* show that the type of tannin was condensed tannin. Principal colouring matter of henna (The lawsone) was identified on the basis of comparison with authentic sample as 2-hydroxy-1,4-naphthoquinone. Leucocyandin, epicatechin, catechin and quercitrin were found identical with that of the authentic samples. The aqueous henna leaves extract show uv λ_{max} at 267 nm. . The spray drying of aqueous henna leaves extract have a fine brown powder with 33-35% yield.

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