Pharmaceutica Analytica Acta
ISSN: 2153-2435

Babili et al., Pharmaceut Anal Acta 2013, 4:1
DOI: 10.4172/2153-2435.1000203

Research Article Open Access

Lawsonia Inermis: Its Anatomy and its Antimalarial, Antioxidant and Human Breast Cancer Cells MCF7 Activities

Fatiha El Babili[1,3]*, Alex Valentin[2] and Christian Chatelain[1]

[1]*Faculté des Sciences Pharmaceutiques de Toulouse-Laboratoire de Botanique-Mycologie-Faculté de Pharmacie, 35 ch. des Maraîchers - F- 31062 Toulouse, France*
[2]*Laboratoire de Pharmacochimie des Substances Naturelles et Pharmacophores Redox, UMR 152, UPS, Université de Toulouse, 118 Route de Narbonne, F-31062 Toulouse Cedex 9, France*
[3]*Laboratoire des Interactions Moléculaires et Réactivité Chimique et Photochimique UMR CNRS 5623, Université de Toulouse, 118 Route de Narbonne, F-31062 Toulouse Cedex 9, France*

Abstract

Anatomical features were highlighted to improve its identification, because although henna has some benefits, it has a wide spectrum of adverse effects from contact dermatitis to severe angioneurotic oedema and haemolysis. Serial extractions in petroleum ether, ethyl acetate, ethanol and water were performed on leaves of *Lawsonia inermis* and were studied. In these extracts, different chemical families were measured such as polyphenols (Gallic acid equivalent 71.7-129.6 g/Kg), tannins (Catechin equivalent 31.3-477.9 g/Kg), antocyanins (Cyanidin equivalent 0.75-5.48 mg/Kg) and flavonoids (Quercetin equivalent 16.2-85.6 g/Kg).

This present study reports ABTS/DPPH assay and antimalarial activity. We also checked activity against human breast cancer cells MCF7.

The best extract with an antioxidant activity (IC_{50}=6.9 ± 0.1 mg/L) was obtained by ethanol. We found an antimalarial activity of the petroleum ether extract (27 mg/L) of henna. Henna extracts showed activity against human breast cancer cells (MCF7) with the ethyl acetate extract (27 mg/L) and petroleum extract (22 mg/L).

Keywords: *Lawsonia inermis*; Antioxidant activity; Antimalarial activity; Cytotoxicity MCF7

Introduction

Lawsonia inermis L. (= L. alba Lamk.), belonging to the Lythraceae family, is a very widespread medicinal plant and natural dye in the world. This herb which has interesting dyeing properties was used traditionally for centuries in Asia, in Africa for dyeing hair and painting skin and nails. Since 1890, it has been widely distributed to Europe and nowadays available in worldwide market as ingredient in many hair dyes and hair-care products. Lawsonia. inermis, also called "henna", contains many kinds of chemical compounds, such as coumarin, quinones, flavonoids etc. and has stronger physiological activity. Due to its natural and harmless characters, the Henna is used as a kind of natural dye, and is used as a raw material for natural hair dyes. In addition, Henna can be used as dyes for textile and tattoo material. The medicinal value of L. inermis is very high and it is used for treatments of headache, jaundice, leprosy [1]. In addition, Henna was shown as an unusual cause of children suicide [2].

The phytochemistry of henna was largely studied and revealed interesting information. Already in 1920 the dye principle was known. Lawsone, $C_{10}H_6O_3$, the colouring matter contained in henna leaves, is fixed well by wool, silk and tenaciously by the skin [3]. In 1973, four fractions with antibacterial activity were isolated by thin-layer chromatography of the ethanol extract of *Lawsonia inermis* leaves. 3 of which were identified as gallic acid [149-91-7], lawsone (2-hydroxy-1,4-naphthoquinone)(I) [83-72-7], and 1,4-naphthoquinone [130-15-4] [4].

Then in 1976, a coumarin, lacoumarin, was isolated from *L. inarmis*. It was then the turn of xanthones; two xanthones isolated from *L. inermis* were characterized as 1,3-dihydroxy-6,7-dimethoxyxanthone and 1-hydroxy-3,6-diacetoxy-7-methoxyxanthone and named laxanthone I and II, respectively. Another xanthone, named laxanthone III was identified from chemical and spectral data [5].

Apigenin-7-glucoside, apigenin-4'-glucoside, luteolin-7-glucoside, and luteolin-3'-glucoside were isolated from leaf extracts of *L. innermis*. Its methanol extract yielded β-sitosterol, stigmasterol and 1,2-dihydroxy-4-glucosyloxynaphthalene. The leaves of *L. inermis* yielded luteolin, acacetin-7-O-glucoside, glucoside of β-sitosterol [6]. Two pentacyclic triterpenes isolated from the bark of *L. inermis* (henna) were identified as 3β,30-dihydroxylup-20(29)-ene (hennadiol) and (20S)-3β,30-dihydroxylupane [6].

In 1992, a sterol, namely lawsaritol, was isolated from the roots of *L. inermis* and elucidated as 24β-ethylcholest-4-en-3β-ol and from the stem bark of *L. inermis* 3-methylnonacosan-1-ol [7]. The methanol extract of the defatted *L. inermis* seeds has led to the isolation of two triterpenoids lawnermis acid and its methyl ester [8]. Two pentacyclic triterpenoids have been isolated from the aerial parts of *Lawsonia alba*. The structures of the new constituents, named as lawsonic acid (I) and lawsonin (II) have been elucidated as 3β-E-ferulyloxy-lup-20(29)-en-28-oic acid and 3β-E-ferulyloxy-urs-11-en-13β-ol respectively, through spectroscopic studies [9].

The biological properties of henna were also studied as follows:

Crude ethanol extract of *Lawsonia inermis* L. produced anti-

***Corresponding author:** Fatiha El Babili, Laboratoire de Botanique-Mycologie, Faculté de Pharmacie, Université de Toulouse, 118 Route de Narbonne, F-31062 Toulouse Cedex 9, France, Tel: +33562259817; E-mail: fatiha.el-babili@univ-tlse3.fr

Received November 26, 2012; **Accepted** January 23, 2013; **Published** January 25, 2013

Citation: Babili FE, Valentin A, Chatelain C (2013) *Lawsonia Inermis*: Its Anatomy and its Antimalarial, Antioxidant and Human Breast Cancer Cells MCF7 Activities. Pharmaceut Anal Acta 4: 203. doi:10.4172/2153-2435.1000203

inflammatory, analgesic, molluscicidal, antibacterial and antipyretic effects. The ethanolic extract of *Lawsonia inermis* leaves and lawsone tested for trypsin inhibitory activity showed an IC_{50} value of 64.87 and 48.6 microg/ml, respectively [10]. Its ethanol leaf extract was active against a wide range of microbes, such as Staphylococcus, Streptococcus, Brucella and Salmonella, but was inactive against *Pseudomonas aeruginosa* and *Candida albicans* [4].

Its ethyl acetate extracts exhibit cytotoxic activity [11]. In fact, Isoplumbagin isolated from the air-dried stem bark of *Lawsonia inermis* exhibited cytotoxic activity (against melanoma and colon cancer cell lines as well as against several of the non-small cell lungs, colon, CNS and renal cell lines) [12].

As we can see, this plant presents strong potential. That is why we seek to complement the knowledge of henna with chemical and biological studies. The aim of our work on Henna was to study to study its anatomical features, the chemical composition of its extracts in the chemicals families such as polyphenols, tannins, antocyanins and flavonoids. The activity of Henna extracts was also evaluated as an antioxidant and anti-malarial. Finally, the toxicity against cancer cells of human breast (MCF7) was also studied for these samples.

Materials and Methods

Plant material

Lawsonia inermis L. collected in Morocco during 2009. Voucher specimens of leaves identified by Professor C. Chatelain and deposited at the herbarium of the Faculty of Pharmacy in Toulouse.

Preparing slides

Observations are based on microscopic studies of sectioned and stained material of tissues.

Transverse sections are prepared with a sliding microtome (MSE) and stained in alun carmine-green combination or *Mirande* reagent [13] during 2 to 3 minutes then washed with water. Following staining, the transverse sections are mounted on glass slides using glycerine gel. Powder observations were made using *Chloral hydrate solution* R [14]. Observations were made with a LEICA Microsystems DMLB microscope, and pictures were taken with Digital Camera Power Shot S40 CANON photo-micrographic system. For the description we have used some help books [15].

Preparation of extracts

Ground leaves from *lawsonia* (80 g), placed in an open column chromatography, was first defatted with petroleum ether (0.8 L) at ambient pressure and temperature. After removal of petroleum ether under reduced pressure, a dried extract obtained as green residue, with an extraction yield of 1.12%. Then, the firstly lixiviate oregano powder was lixiviate with ethyl acetate (0.8 L). After removal of ethyl acetate under reduced pressure, a dried extract was obtained as white powder, slightly coloured in yellow, with an extraction yield of 1.23%. Finally, the last lixiviation was made with ethanol (1.2 L). After removal of ethanol under reduced pressure, a dried extract obtained as brown powder with an extraction yield of 15%. For the decoction, 50 g of henna powder were extracted with boiling water (0.5 L) during 30 min. After removal of water by lyophilisation, brown powder was obtained with an extraction yield of 12.2%.

Chemicals

All chemicals used were of analytical reagent grade. All reagents purchased from Sigma-Aldrich, Fluka (Saint-Quentin France).

Total amount of phenolic compounds

The total phenolic amount of each extract was determined by the Folin-Ciocalteu method [16]. A diluted solution of each extract (0.5 mL) was mixed with Folin Ciocalteu reagent (0.2 N, 2.5 mL). This mixture was allowed to stand at room temperature for 5 min and then sodium carbonate solution (75 g/L in water, 2 mL) was added. After 1 h of incubation, the absorbance was measured at 765 nm against blank using a Helios spectrophotometer (Unicam, Cambridge, UK). A standard calibration curve was plotted using gallic acid (0-300 mg/L). Results were expressed as g of gallic acid equivalents (GAE)/Kg of dry mass.

Condensed tannin content

Catechins and proanthocyanidins reactive with vanillin were analyzed by the vanillin method [17]. One milliliter (1 mL) of each extract solution was mixed in a test tube with 2 mL of vanillin (1% in 7 M H_2SO_4) in an ice bath. Then the mix was incubated at 25°C. After 15 minutes, the solution absorbance was read at 500 nm. Concentrations were calculated as g catechin equivalents (CE)/Kg dry mass from a calibration curve.

Total flavonoids determination

The total flavonoids were estimated according to the Dowd method as adapted by Arvouet-Grand [18]. A diluted methanolic solution (4 mL) of each extract was mixed with a solution (4 mL) of aluminium trichloride ($AlCl_3$) in methanol (2%). The absorbance was read at 415 nm after 15 minutes against a blank sample consisting of a methanol (4 mL) and extract (4 mL) without $AlCl_3$. Quercetin was used as reference compound to produce the standard curve, and the results were expressed as g of quercetin equivalents (QE)/Kg of dry mass.

Determination of total anthocyanin content

Total anthocyanin content was measured with the pH differential absorbance method, as described by Cheng and Breen [19]. Briefly, absorbance of the extract was measured at 510 and 700 nm in buffers at pH 1.0 (hydrochloric acid-potassium chloride, 0.2 M) and 4.5 (acetate acid-sodium acetate, 1 M). The wavelength reading was performed after 15 minutes of incubation. Anthocyanin content was calculated using a molar extinction coefficient (ε) of 29600 (cyanidin-3-glucoside) and absorbance of $A=((A_{510}-A_{700})_{pH\ 1.0}-(A_{510}-A_{700})_{pH\ 4.5})$. Results were expressed as mg cyanidin-3-glucoside equivalent (C3GE) /Kg of dry mass.

Free radical scavenging activity: DPPH test

Antioxidant scavenging activity was studied using 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) as described by Blois [20] with some modifications; 1.5 mL of various dilutions of the test materials (essential oil or plant extracts) were mixed with 1.5 mL of a 0.2 mM methanolic DPPH solution. After an incubation period of 30 minutes at 25°C, the absorbance at 520 nm, the wavelength of maximum absorbance of DPPH, were recorded as $A_{(sample)}$, using a Helios spectrophotometer (Unicam, Cambridge, UK). A blank experiment was also carried out applying the same procedure to a solution without the test material and the absorbance was recorded as $A_{(blank)}$. The free radical-scavenging activity of each solution was then calculated as percent inhibition according to the following equation:

$$\% \text{ inhibition} = 100\ (A_{(blank)} - A_{(sample)})/A_{(blank)}$$

Antioxidant activity of essential oil or extracts was expressed as

IC_{50}, defined as the concentration of the test material required to cause a 50% decrease in initial DPPH concentration. Ascorbic acid was used as a standard. All measurements were performed in triplicate.

ABTS radical-scavenging assay

The radical scavenging capacity of the samples for the ABTS (2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonate) radical cation was determined as described by Re et al. [21]. ABTS was generated by mixing a 7 mM of ABTS at pH 7.4 (5 mM NaH_2PO_4, 5 mM Na_2HPO_4 and 154 mM NaCl) with 2.5 mM potassium persulfate (final concentration) followed by storage in the dark at room temperature for 16 h before use. The mixture was diluted with ethanol to give an absorbance of 0.70 ± 0.02 units at 734 nm using a spectrophotometer. For each sample, diluted methanol solution of the sample (100 µL) was allowed to react with fresh ABTS solution (900 µL), and then the absorbance was measured 6 minutes after initial mixing. Ascorbic acid was used as a standard and the capacity of free radical scavenging was expressed by IC_{50} (mg/L) values calculated, denoting the concentration required to scavenge 50% of ABTS radicals. The capacity of free radical scavenging IC_{50} was determined using the same previously used equation for the DPPH method. All measurements were performed in triplicate.

Assays on *Plasmodium falciparum in vitro*

P. falciparum FcB1-Columbia strain (chloroquine-resistant: IC_{50} for chloroquine: 186 nM) was cultured continuously according to Trager and Jensen [22] with modifications described by Benoit et al. [23]. The IC_{50} value for chloroquine was checked every 2 months, and we observed no significant variations. The parasites were maintained *in vitro* in human red blood cells (O±; EFS; Toulouse, France), diluted to 4% hematocrit in RPMI 1640 medium (Lonza; Emerainville, France) supplemented with 25 mM Hepes and 30 mM $NaHCO_3$ and complemented with 7% human AB+ serum (EFS). Parasites cultures were synchronized by D-sorbitol lysis (5% of D-sorbitol in sterile water; D-sorbitol) as reported by Lambros and Vanderberg [24]. The antiplasmodial activities (essential oil, plant extracts) were evaluated by a radioactive micromethod derived from that of Desjardins et al. [25] with the modifications reported by Munoz et al. [26]. Tests were performed in triplicate in 96-well culture plates (TPP) with cultures mostly at ring stages (synchronisation interval, 16 h) at 0.5-1% parasitemia (hematocrit, 1.5%). Parasite culture was incubated with each sample at growing dilution for 48 h. Parasite growth was estimated by (^{3}H)-hypoxanthine (Perkin-Elmer; Courtaboeuf, France) incorporation, which was added to the plates 24 h before freezing. After 48 h incubation, plates were frozen-defrosted and each well was harvested on a glass fiber filter. Incorporated (^{3}H)-hypoxanthine was then determined with a betacounter (1450-Microbeta Trilux; Wallac-Perkin Elmer). IC_{50} values were determined by linear least square regression analysis. The control parasite culture, free from any sample, was referred to as 100% growth. IC_{50} were determined graphically in concentration versus percent inhibition curves. Chloroquine diphosphate was used as positive control. The antimalarial activity of samples was expressed as their 50% inhibitory concentrations (IC_{50}), representing the concentration of drug that induced a decrease of 50% in the parasitaemia compared to the positive control culture referred to as 100% parasitaemia. The antimalarial activities of plant extracts can be classified as follow: very active if IC_{50}<5 mg/L, active if IC_{50} between 5 and 50 mg/L, weakly active if IC_{50} between 50 and 100 mg/L and inactive if IC_{50}>100 mg/L [27]. For each sample, the CAR (cytotoxic/antimalarial ratio) was calculated. It indicated the specificity of the antiplasmodial activity.

Evaluation of the cytotoxicity against human breast cancer cells MCF7

Sample cytotoxicity was studied on MCF7, a human breast cancer cell line. The cells were cultured in the same conditions as those used for *P. falciparum*, except for the 10% human serum, which was replaced by 10% foetal calf serum (Lonza). For the determination of cytotoxicity, cells were distributed in 96-well plates at 3×10^4 cells/well in 100 µL, and then 100 µL of culture medium containing samples at various concentrations were added. Cell growth was estimated by (^{3}H)-hypoxanthine incorporation after 48 h incubation exactly as for the *P. falciparum* assay. The (^{3}H)-hypoxanthine incorporation in the presence of sample was compared with that of control cultures without sample (positive control being doxorubicin) [28].

Statistical analysis

All data were expressed as means ± standard deviations of triplicate measurements. The confidence limits were set at $P<0.05$. Standard deviations (SD) did not exceed 5% for the majority of the values obtained.

Results and Discussion

Anatomical study of *Lawsonia inermis* shows the following elements.

In stem

Lawsonia inermis transverse section, stained with *Mirande* reagent shows various characteristic elements from the exterior to the interior.

An epidermis, with striated cuticle, made up of one layer of isodiametric cellulose thin-walled cells - A Phelloderm made up of a layer of cellulose thin-walled cells - A Cortical parenchyma made up of rounded thin-walled cells separated by meatuses and containing crystals of calcium oxalate. Its internal zone is invaded with laticiferous surrounded of acicular crystals of calcium oxalate. A pericycle zone made up of lignified thick-walled fibres clusters with narrow lumen - A continuous conducting ring made up of secondary phloem composed of cellulose tabular thin-walled-cells and wood composed of vessels, often aligned radially, and strongly lignified woody parenchyma. A PITH made up of polyhedral slightly sclerified thick-walled cells separated by meatuses, invaded by clusters of crystals of calcium oxalate (Figures 1-4).

In leaf

Lawsonia inermis (Figures 5 and 6) leaf transverse section, stained with *Mirande* reagent shows various characteristic elements from the superior face to the inferior face.

MIDRIB: An superior epidermis, with striated cuticle, made up of one layer of isodiametric thin-walled cells- A parenchyma made up of cellulose rounded thin-walled cells containing by place crystals of calcium oxalate. An arc shaped with vascular tissue composed with woody bundle and secondary phloem - An inferior cuticularized with anomocytic stomata, made up of one layer of cellulose isodiametric thin-walled cells;

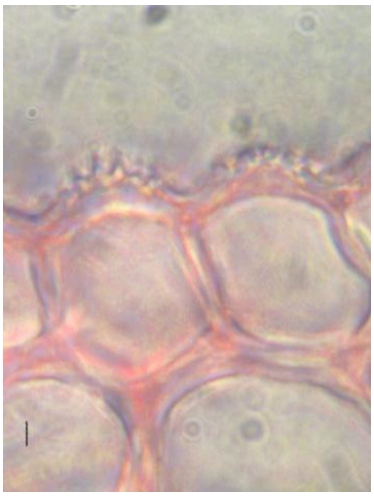
LAMINA: A superior stomatiferous epidermis, with striated cuticle, made up of one layer of isodiametric (Figure 5), cellulosic thin-walled cells – An asymmetrical heterogeneous mesophyll made up of palisade and spongy parenchyma. In the middle, there are secondary vascular tissues with crystals of calcium oxalate (Figure 6). An inferior

cuticularized epidermis, with anomocytic stomata, made up of one layer of cellulose isodiametric thin-walled cells;

Sampling of the inferior epidermis shows anomocytic stomata (Figure 7).

Chemical composition

As far as we know, this work is the first time that the chemical composition of aerial part of *lawsonia inermis* was evaluated (Table 1). Total amount of flavonoids, in henna ethyl acetate extract, was the higher (85.6 ± 3.1 g/kg equivalent Quercetin), while ethyl acetate extract was the richest on polyphenols (129.6 ± 4.1 g/kg eq Gallic acid). The antocyanins (5.48 ± 0.17 mg de Cyaniding/kg) were present in higher content in ethanol extract. The tannins exist in all extracts with an amount between 31.3 ± 0.8 (decoction) to 477.9 ± 12.9 (ethyl acetate extract) eq Catechin (g/Kg dry). The chemical composition study of henna extracts allows us to begin to understand its properties, pending clarifies this work by a structure-activity relationship.

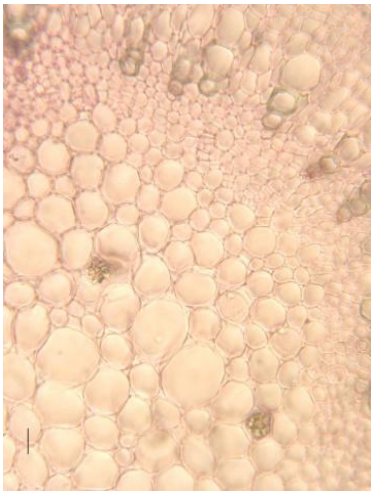


(× 100)

Figure 1: Stem epidermis: Striated cuticle (SC), Epidermis (E).

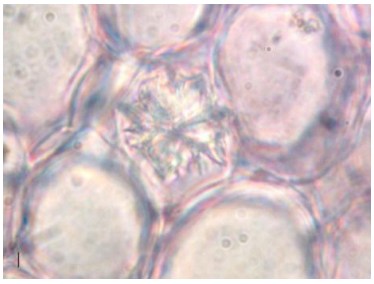
(× 20)

Figure 2: Vascular bundle: Cortical parenchyma (CP) – Phloem (PH) - Wood (W).



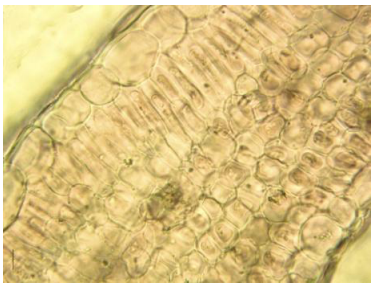
(× 20)

Figure 3: Pith: Medullar parenchyma invaded by calcium oxalate crystals.



(× 100)

Figure 4: Stem: Crystals of calcium oxalate.

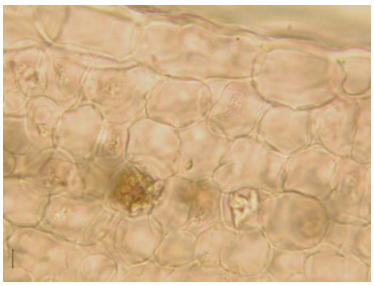


(× 20)

Figure 5: Lamina.

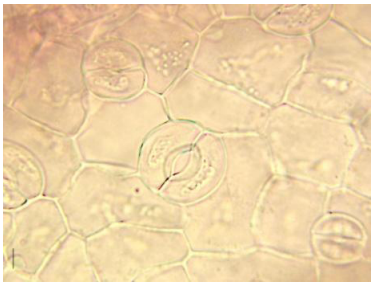
Antioxidant capacity

In our study, the henna antioxidant activity (Table 2) showed for ethyl acetate extract an IC_{50} of 29.5 ± 0.8 mg/L in DPPH radical scavenging assay and IC_{50} of 8.6 ± 0.2 mg/L in ABTS radical scavenging assay. The ethanol extract exhibited an IC_{50} of 14.1 ± 0.5 mg/L in DPPH and with IC_{50} of 6.9 ± 0.1 mg/L in ABTS radical scavenging assay. Petroleum ether extract was the less antioxidant extract. The decoction



(× 40)

Figure 6: Lamina details: Crystals of calcium oxalate between palisade and spongy mesophyll.



(× 40)

Figure 7: Sampling of inferior epidermis: Anomocytic stomata. Scale bars: (x 20): 2.3 μm, (x 40): 0.5 μm, (x 100): 0.07 μm.

Type of extract	Polyphenols (eq Gallic acid)[a]	Tannins (eq Catechin)[a]	Flavonoids (eq Quercetin)[a]	Anthocyanins (eq cyanindin)[b]
Ethyl acetate	129.6 ± 4.1	477.9 ± 12.9	85.6 ± 3.1	0.75 ± 0.02
Petroleum ether	71.7 ± 2.1	315.6 ± 11.2	52.9 ± 1.9	1.98 ± 0.06
Ethanol	105.8 ± 4.2	58.1 ± 1.7	33.8 ± 1.4	5.48 ± 0.17
Decoction	100.2 ± 3.5	31.3 ± 0.8	16.2 ± 0.5	1.86 ± 0.05

[a]: g/Kg dry; [b]: mg/Kg dry

Table 1: Chemical composition of *Henna* extracts.

Type of extract	ABTS assay IC_{50} (mg/L)	DPPH assay IC_{50} (mg/L)
Ethyl acetate	8.6 ± 0.2	29.5 ± 0.8
Petroleum ether	738.7 ± 9.6	161.6 ± 2.3
Ethanol	6.9 ± 0.1	14.1 ± 0.5
Decoction	16.8 ± 0.7	13.0 ± 0.6
Vit C	1.9 ± 0.1	4.4 ± 0.2

Table 2: Henna extracts antioxidant activity.

Type of extract	Cytotoxic activity IC_{50} (mg/L)	Antimalarial activity IC_{50} (mg/L)
Petroleum ether	22	27
Ethyl acetate	27	33
Ethanol	34	53
Chloroquine	ND	0.031
Doxorubicin	0.218	ND

Table 3: Biological activity of Henna extracts.

was the most antioxidant extract with IC_{50} of 13.0 ± 0.6 mg/L in DPPH and with IC_{50} of 16.8 ± 0.7 mg/L in ABTS radical scavenging assay. Ethanol extract seems more active than decoction in ABTS radical scavenging assay, but the decoction was so strong in DPPH radical scavenging assay. These two forms were used in traditional medicine.

Given its ease of use, this result confirms the traditional use of "henna" in Morocco, thus highlighting its potential in health.

Cytotoxicity and antimalarial activities

We found an antiplasmodial activity (Table 3) against both FcB1-Columbia and FcM29-Cameroon strains of *P. falciparum* of the petroleum ether extract (27 mg/L) and ethyl extract (33 mg/L) of Henna. Which places our Henna extracts in the range of plants showing an interesting activity, knowing that beyond an IC_{50} of the 35.5 mg/L antimalarial activity is low. However, this is the first time we tested the antimalarial activity of Henna.

Henna showed, also for the first time an activity against human breast cancer cells (MCF7) of the ethyl acetate extract (27 mg/L) and petroleum extract (22 mg/L). Comparison of the plant extracts activity with those of chloroquine does not indicate that the bioactive compounds have a low activity, but were dilute in the whole extracts.

Because of antimalarial, antioxidant and cytotoxic activities of its extracts, *L. Inermis* is an interesting natural resource for cosmetic industry. In conclusion, our study can be considered as a report on the anatomical features and on antimalarial and antioxidant properties of extracts prepared from Henna. The present results may explain the traditional medicinal use of henna in Morocco. It seems interesting to isolate the molecules responsible for these potentials.

References

1. Lei G, Wang, Yi-jun, Yang, Zhao-xia (2004) College of Chemical Engineering, Qingdao University, Qingdao, Peop Rep China. Qingdao Daxue Xuebao, Gongcheng Jishuban 19: 37-42.
2. Kok AN, Ertekin V, Bilge Y, Işik AF (2005) An unusual cause of suicide: henna (Lawsonia inermis Linn.). J Emerg Med 29: 343-344.
3. Tommasi G (1920) Henna (Lawsonia inermis). Chemical constitution of lawsone. II - Gazzetta Chimica Italiana 50: 263-272.
4. Abd-el-Malek Y, El-Leithy MA, Reda FA, Khalil M (1973) Antimicrobial principles in leaves of Lawsonia inermis. Landwirtschaftliche und Technische Mikrobiologie 128: 61-67.
5. Bhardwaj DK, Jain RK, Jain BC, Mehta CK (1978) 1 Hydroxy 3,7 dimethoxy 6 acetoxyxanthone, a new xanthone from Lawsonia inermis. J Agric Food chem 17: 1440-14411.
6. Chakrabartty, Tarakeswar, Poddar, Gurudas St, Pyrek, et al. (1982) Constituents of Indian medicinal plants. Phytochemistry (Elsevier) 21: 1814-1816.
7. Gupta, Sarita, Ali M, Alam M, Sarwar (1992) 24β-Ethylcholest-4-en-3β -ol from the roots of Lawsonia inermis. Phytochemistry 31: 2558-2560.
8. Handa, Geeta, Kapil, Aruna, Sharma, et al. (1997) Lawnermis acid: a new anticomplementary triterpenoid from Lawsonia inermis seeds. Indian Journal of Chemistry 28: 252-256.
9. Siddiqui BS, Kardar MN (2001) Triterpenoids from Lawsonia alba. Phytochemistry 58: 1195-1198.
10. Yogisha S, Samiulla DS, Prashanth D, Padmaja R, Amit A (2001) Trypsin inhibitory activity of Lawsonia inermis. Fitoterapia 73: 690-691.
11. Ali NA, Jülich WD, Kusnick C, Lindequist U (2001) Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. J ethnopharmacology 74: 173-179.
12. Ali M, Grever MR (1998) A cytotoxic naphthoquinone from Lawsonia inermis. Fitoterapia 69: 181-183.
13. Mirande R (1920) Sur le carmin aluné et son emploi, combiné avec celui du vert d'iode, en Histologie végétale. CR Acad Sci 170: 197-199.
14. European Pharmacopoeia (in force), Ed. Maison Neuve SA, Moulins, les Metz.

15. Speranza A, Calzoni GL (2005) Atlas de la structure des plantes Ed. Belin 125-203.

16. Folin O, Ciocalteu V (1927) On tyrosine and tryptophane determination in proteins. J Biol Chem 73: 627-650.

17. Naczk M, Amarowicz R, Pink D, Shahidi F (2000) Insoluble condensed tannins of canola/rapeseed. J Agric Food Chem 48: 1758-1762.

18. Arvouet-Grand A, Vennat B, Pourrat A, Legret P (1994) Standardisation d'un extrait de propolis et identification des principaux constituants. J Pharm Belg 49: 462-468.

19. Cheng GW, Breen PJ (1992) Cell count and size in relation to fruit size among strawberry cultivars. J Am Soc Hort Sci 117: 946-950.

20. Blois MS (1958) Antioxidant determinations by the use of a stable free radical. Nature 181: 1199-1200.

21. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, et al. (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 26: 1231-1237.

22. Trager W, Jensen JB (1976) Human malaria parasites in continuous culture. J Parasitol 19: 484-486.

23. Benoit F, Valentin A, Pelissier Y, Diafouka F, Marion C, et al. (1996) In vitro antimalarial activity of vegetal extracts used in West African traditional medicine. Am J Trop Med Hyg 54: 67-71.

24. Lambros C, Vanderberg JP (1979) Synchronization of Plasmodium falciparum Erythrocytic Stages in culture. J Parasitol 65: 418-420.

25. Desjardins RE, Canfield CJ, Haynes JD, Chulay JD (1979) Quantitative assessment of antimalarial activity in vitro by a semiautomated microdilution technique. Antimicrob Agents Chemother 16: 710-718.

26. Munoz V, Sauvain M, Mollinedo P, Callapa J, Rojas I, et al. (1999) Antimalarial activity and cytotoxicity of (-)-roemrefidine isolated from the stem bark of Sparattanthelium amazonum. Planta Med 65: 448-449.

27. Ouattara Y, Sanon S, Traoré Y, Mahiou V, Azas N, et al. (2006) Antimalarial activity of Swartzia madagascariensis desv. (Leguminosae), Combretum glutinosum Guill. & Perr. (Combretaceae) and Tinospora Bakis Miers. (Menispermaceae), Burkina Faso Medicinal Plants. Afr J Trad Cam Alt Med 3: 75-81.

28. Valentin A, Benoit-Vical F, Moulis C, Stanislas E, Mallie M, et al. (1997) In vitro antimalarial activity of penduline, a bisbenzylisoquinoline from Isopyrum thalictroides. Antimicrob Agents Chemother 41: 2305-2307.