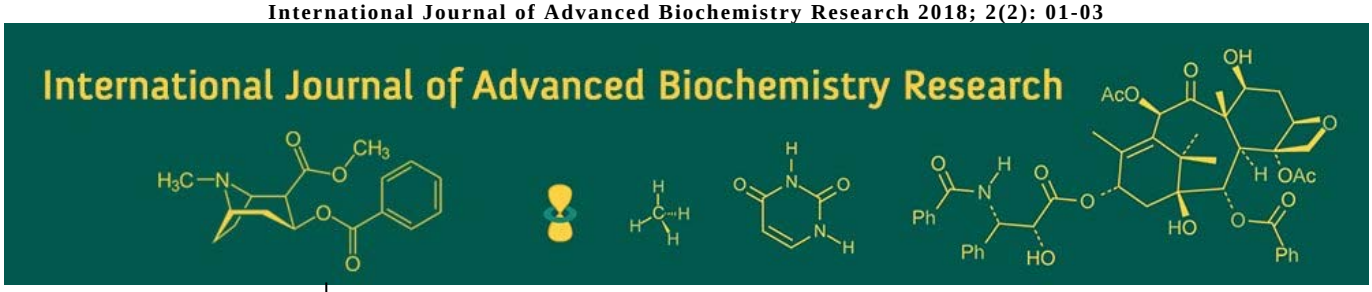

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Chemical composition of *Lawsonia inermis* cultivated under Sudanese conditions-existence of chemotypes species

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Abstract
Objectives: The objectives of this study are to: (i) qualitatively and quantitatively assessing the chemical components of *Lawsonia inermis* Leaves from cultivated plants in two localities in Central Sudan, and (ii) to identify of their chemotypes by using GC/MS.
Materials and Methods: The cultivated plant *Lawsonia inermis* L. in Ed-Damer and AL-Fetaehab regions were collected in October 2014 from Ed-Damer city, Nile River State (Ed-Damer sample) and Omdurman South, Khartoum State (AL-Fetaehab sample). All plant samples under study were separately extracted by ethanol (80%) at room temperature for 24 hours. After extraction it were filtered and then the removal of solvent was done. The qualitative and quantitative analysis of the samples were carried out by using GC/MS technique. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST).
Results: The ethanol extract of leaves of L. *inermis* (AL-Fetaehab sample) showed that the D-allose (17.61%), lawsone (12.87%), beta-D-glucopyranoside, methyl (12.74%), phytol (10.78%), 1-isobutoxy-1-methoxypropane (9.18%), n-hexadecanoic acidin6.33%), 9,12,15-octadecatrienoic acid (Z,Z,Z) (4.44%), squalene (4.06%) and vitamin E (3.60%) as the major phytochemical constituents, whereas the ethanol extract of leaves of L. *inermis* (Ed-Damer sample) showed that the compounds: beta-D-glucopyranoside, methyl (36.10%), phytol (10.85%), 9,12,15-Octadecatrienoic acid, Z,Z,Z (7.31%), squalene (7.20%), vitamin E (6.82%) and lawsone (6.79%) as the major phytochemical constituents.
Conclusion: According to the chemical composition of *Lawsonia inermis*, it can be classified as (i) lawsone (naphtha Quinone) Chemotype (AL-Fetaehab sample) and (ii) ethyl alpha-d-glucopyranoside (Phenolic glucoside) Chemotype (Ed-Damer sample).

Keywords: *Lawsonia inermis*, chemical composition, lawsone, ethyl alpha- d-glucopyranose, Central Sudan

1. Introduction
Lawsonia inermis L. (Family: Lythraceae) is a natural red colouring agent, commonly named "Henna" and is cultivated in the savannah region. Leaves are commonly used as cosmetics for staining skin, hair and wool (Gallo *et al.*, 2008; Takeda and Fatope, 1988) [1, 2]. It considered as a valuable source of unique natural products for development of medicines against various diseases and also for the development of industrial products (Chaudhary *et al.*, 2010) [3].
Lawsone, a major colorant component extracted from *Lawsonia inermis* (Henna), naphthaquinone-type dye. This compound is well known from its cosmetic use to dye hair, nails and skin while it is been used to colour textiles including leather, wool and silk (Endrini 2002) [4]. Lawsone found to possess antibacterial, antifungal, antiviral and antineoplastic activities. They could also inhibit tumor cell growth (Endrini *et al.*, 2002) [4].
Henna (*Lawsonia inermis* L.) has been known as the natural source of dye, besides having medicinal properties. Henna powder is made into paste in water and used for skin decorations and hair dyeing. The use of henna in Sudan goes back to the ancient times as a soothing agent and also for dyeing material. The objectives of this study are to: (i) qualitatively and quantitatively assessing the chemical components of *Lawsonia inermis* Leaves from cultivated plants in two localities in Central Sudan, and (ii) identification of their chemotypes using GC/MS.

2. Materials and Methods

2.1 Plant Material

The cultivated plant *Lawsonia inermis* L. in Ed-Damer and AL-Fetaehab regions were collected in October 2014 from Ed-Damer city, Nile River State and Omdurman South, Khartoum State. The plant materials were taxonomically identified by one author, Prof. HH EL-Kamali, Department of Botany, Faculty of Science and Technology, Omdurman Islamic University.

2.2 Sample preparation

Each plant sample was carefully dried in the Botany laboratory at Omdurman Islamic University at room temperature. The plant materials were then grounded to fine powder with a mechanical Miller and the powder was kept in glass container at room temperature.

2.3 Plant Extraction

All plant samples under study were separately extracted by ethanol (80%) at room temperature for 24 hours. After extraction it were filtered and then the removal of solvent was done.

2.4 Chromatography Mass Spectrometer (GC/MS) conditions

The qualitative and quantitative analysis of the samples were carried out by using GC/MS technique model (GC/MS – QP2010-Ultra) from Japan (Shimadzu Company) with serial number 020525101565SA) and capillary column (Rtx-5ms- 30mx0.25mm x0.25 um). The sample was injected by using split mode, instrument operating in EI mode at 70 eV. Helium as the carrier gas passed with flow rate 1.69 ml/min. The temperature program was started from 50 C with rate 7C/min to 18C then the rate was changed to 10C/min reaching 300C as final temperature degree, with 2 minutes as hold time, the injection port temperature was 300C, the ion source temperature was 200 C and the interface temperature was 250C. The sample was analyzed by using scan mode in the range of m/z 40-500 charges to ratio and the total run time was 30 minutes.

2.4.1 Identification of components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

3. Results and Discussion

3.1 GC/MS analysis of *Lawsonia inermis* (AL-Fetaehab sample)

GC/MS chromatogram of ethanol extract of leaves of L. *inermis* (AL-Fetaehab sample) (Figure 1) showed the presence of 27 compounds. The Table 1 shows the compounds: D-allose (17.61%), lawsone (12.87%), beta-D-glucopyranoside, methyl (12.74%), phytol (10.78%), 1-isobutoxy-1-methoxypropane (9.18%), n-hexadecanoic acid (6.33%), 9,12,15-octadecatrienoic acid (Z,Z,Z) (4.44%), squalene (4.06%) and vitamin E (3.60%) as the major phytochemical constituents.

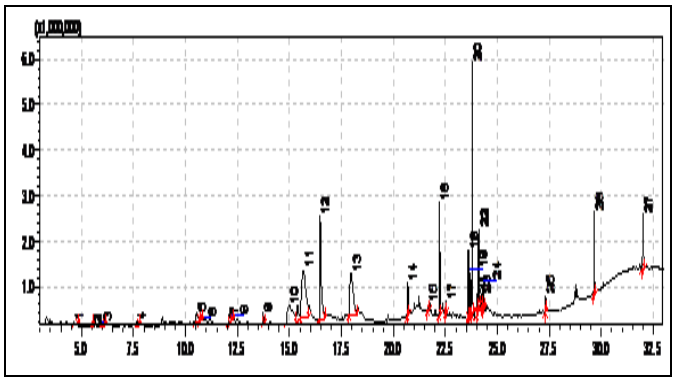

Fig 1: The GC/MS chromatogram of ethanol extracts of leaves of *L. inermis* (AL-Fetaehab sample)

Table 1: Major chemical compounds identified in ethanolic extracts of L. *inermis* (AL-Fetaehab sample)

Peak #	Compounds	%
11	D-allose	17.61
12	Lawsone	12.87
13	beta-D-glucopyranoside, methyl	12.74
20	Phytol	10.78
10	1-isobutoxy-1-methoxypropane	9.18
16	n-hexadecanoic acid	6.33
22	9,12,15-octadecatrienoic acid (Z,Z,Z)	4.44
26	Squalene	4.06
27	vitamin E	3.60

GC/MS analysis of *Lawsonia inermis* (Ed-Damer sample)

GC/MS chromatogram of ethanol extract of leaves of L. *inermis* (Ed-Damer sample) (Figure 2) showed the presence of 28 compounds. The Table 2 shows the compounds: beta-D-glucopyranoside, methyl (36.10%), phytol (10.85%), 9,12,15-Octadecatrienoic acid, Z,Z,Z (7.31%), squalene (7.20%), vitamin E (6.82%) and lawsone (6.79%) as the major phytochemical constituents.

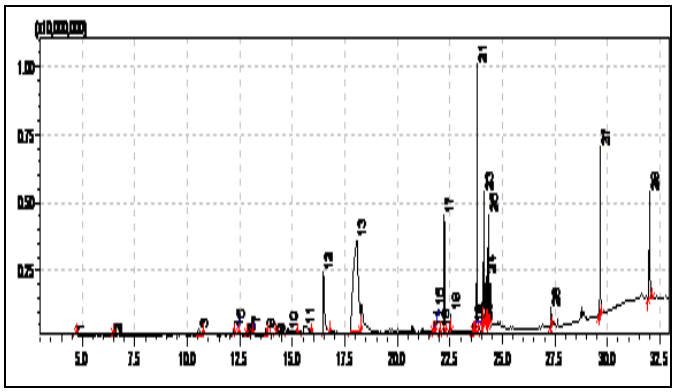

Fig 2: The GC/MS chromatogram of ethanol extracts of leaves of *L. inermis* (Ed-Damer sample)

Table 2: Major chemical compounds identified in ethanolic extracts of L. *inermis* (Ed-Damer sample)

Peak #	Compounds	%
13	beta-D-glucopyranoside, methyl	36.10%
21	Phytol	10.85%
23	9,12,15-Octadecatrienoic acid, Z,Z,Z	7.31%
27	Squalene	7.20%),
28	vitamin E	6.82%
12	Lawsone	6.79%

Ten fatty acids compounds were identified in the ethanolic extract of *Lawsonia inermis* (Ed-Damer sample) (24.43%). The results revealed that 9,12,15-Octadecatrienoic acid (Z,Z,Z) (7.31%) followed by n-hexacdecanoic (6.31%). Ten fatty acids compounds were identified in the ethanolic extract of *Lawsonia inermis* (AL-Fetaehab sample) (21.89%). The results revealed that n-hexacdecanoic (6.33%) was the major component followed by 9,12,15-Octadecatrienoic acid (Z,Z,Z) (4.44%)

Lawsonia inermis (Ed-Damer sample) contain lidocaine (0.15%) whereas no alkaloids in henna AL-Fetaehab sample. Isophytol was found in *L. inermis* Ed-Damer sample (0.18%). The highest lawsone content was found in the L. *inermis* (AL-Fetaehab sample (12.87%).

In previous reports on the chemical content of *Lawsonia inermis* from around the world (Hema *et al.* [5]., 2010, Wagini *et al.* [6]., 2014, Kidanemarium *et al.* [7]., 2013, Mahkam *et al.* [8]., 2014, Upadhyay *et al.* [9]., 2010, Charoensup *et al.* [10]., 2017 and Bakkali *et al.* [11]., 1997), were found in the range between 0.004 and 19.19%. In our results the lawsone content were in average 13.23%.

According to the chemical composition of *Lawsonia inermis*, it can be classified as lawsone (Naphthaquinone) chemotype (AL-Fetaehab sample) and ethyl alpha-d-glucopyranoside (Phenolic glucoside) chemotype (Ed-Damer sample).

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